Early Impairment of Long-Term Depression in the Perirhinal Cortex of a Mouse Model of Alzheimer's Disease

Francesco Tamagnini,¹ Costanza Burattini,¹ Tiziana Casoli,² Marta Ballelli,^{2,3}
Patrizia Fattorelli,^{2,3} and Giorgio Aicardi^{1,4}

Abstract

Visual recognition memory is early impaired in Alzheimer's disease. Long-term depression of synaptic transmission in the perirhinal cortex is critically involved in this form of memory. We found that synaptic transmission was impaired in perirhinal cortex slices obtained from 3-month-old Tg2576 mice, and that 3,000 pulses at 5 Hz induced long-term depression in perirhinal cortex slices from age-matched control mice, but not in those from Tg2576 mice. To our knowledge, these data provide the first evidence of synaptic transmission and long-term depression impairment in the perirhinal cortex in an animal model of Alzheimer's disease, and the earliest synaptic deficit in Tg2576 mice.

Introduction

ALZHEIMER'S DISEASE (AD) IS THE most common form of dementia in the elderly, accounting for 50%–60% of all cases. As life expectancy increases, the number of AD cases worldwide is expected to increase from 35.6 million people living with dementia worldwide in 2010 to 65.7 million by 2030 and 115.4 million by 2050.¹ The cellular hallmarks underlying the pathogenesis of AD are extracellular amyloid- β ($A\beta$) plaque deposition and intracellular accumulation of hyperphosphorylated microtubule-associated tau protein aggregated in neurofibrillary tangles, resulting in synaptic dysregulation and neurodegeneration.² According to the amyloid hypothesis, the progressive accumulation of soluble $A\beta_{40-42}$ oligomers results in cholinergic dysfunction and mild cognitive impairment in the first stages of the disease,³ and later in $A\beta$ plaque deposition, neurofibrillary tangle accumulation, chronic inflammation, neurodegeneration, and consequent severe dementia.⁴

A major goal of AD research is to characterize the mechanisms leading to early functional impairments to improve diagnostic and therapeutic tools. Indeed, transgenic animal models have provided very valuable insights into specific aspects of AD pathogenesis.⁵ Tg2576 is one of the most widely used mouse transgenic lines in this field. It carries the human allele for $A\beta$ precursor protein ($A\beta$ PP) bearing the

Swedish mutation (Lys-670-Asn; Met-671-Leu)⁶ and resulting in high levels of soluble $A\beta$ oligomers in hippocampus and associated structures at 3 months.⁷ Tg2576 mice also show impairments in hippocampal long-term potentiation (LTP) of synaptic transmission, in contextual fear conditioning,⁸ and in visual recognition memory⁹ at 5 months.

The perirhinal cortex (Prh) is a multimodal associative cortex of the temporal lobe that is critically involved in visual recognition memory.¹⁰ Both LTP and long-term depression (LTD) of glutamatergic synaptic transmission have been observed in this brain area, but LTD rather than LTP is considered the cellular correlate of this form of memory.^{11,12} Because cholinergic neurotransmission plays a pivotal role in both visual recognition memory and Prh LTD,¹¹ it is not surprising that impairments in this form of memory begin in the early stages of AD.¹³

The study reported here evaluated if basal synaptic transmission and LTD in Prh of Tg2576 mice is already compromised at 3 months of age.

Materials and Methods

Animals

APP-Swe (Tg2576) transgenic mice and transgene-negative littermates (Taconic Farms, Inc., Hudson, NY) were used. The animals were treated in accordance with the

¹Department of Human and General Physiology, University of Bologna, Bologna, Italy.

²Neurobiology of Aging Laboratory and ³Cellular Bioenergetics Laboratory, INRCA Scientific Technological Area, Ancona, Italy.

⁴Interdepartmental Centre "Luigi Galvani" for the Study of Biophysics, Bioinformatics and Biocomplexity, University of Bologna, Bologna, Italy.

European Community guidelines on animal care, and the experimental protocols were approved by the ethical committee of the University of Bologna. They were housed 1 animal per cage, under controlled environmental conditions (constant temperature [$24^{\circ}\text{C} \pm 1^{\circ}\text{C}$] and humidity [$50\% \pm 5\%$]) and maintained on a 12-hr light/dark cycle and fed *ad libitum*.

Slice preparation

At 3 months of age, each animal was anesthetized with halothane and sacrificed. The brain was rapidly removed and placed in ice-cold ($0\text{--}4^{\circ}\text{C}$), oxygenated (95% $\text{O}_2/5\% \text{CO}_2$) cryoprotective artificial cerebrospinal fluid (aCSF; 124 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4 , 10 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 26 mM NaHCO_3 , and 10 mM glucose, pH 7.4). The rostral and caudal parts of the brain were removed by single scalpel cuts at $\sim 45^{\circ}$ to the dorsoventral axis. The sample was then glued on a stainless steel stage, rostral side up, and immediately placed in the slicing chamber of a vibratome (WPI Europe, Berlin, Germany) filled with ice-cold, oxygenated cryoprotective aCSF. Semicoronal slices ($400 \mu\text{m}$ thickness) comprising hippocampus, Prh, and lateral entorhinal cortex were obtained and then left to recover for at least 60 min in oxygenated aCSF (in 124 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4 , 1 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2 mM CaCl_2 , 26 mM NaHCO_3 , and 10 mM glucose, pH 7.4) at room temperature. After recovery, one slice was placed in a submerged recording chamber and perfused continuously (3 mL/min) with oxygenated aCSF maintained at 32°C .

Electrophysiological recordings

After at least 30 min, square current pulses (duration, 0.2 msec) were applied every 30 sec (0.033 Hz) by using a stimulus generator (Master8, AMPI, Israel) connected through a stimulus isolation unit to a concentric bipolar electrode (40–80 K Ω) positioned in layers II–III on the temporal side of the rhinal sulcus. Evoked extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded with a glass micropipette pulled on a horizontal puller (Sutter Instruments, Novato, CA) with a resistance of 3–5 M Ω , filled with 2 M NaCl solution, inserted in layers II–III in the rhinal sulcus at $\approx 500 \mu\text{m}$ from the stimulation electrode, and connected to a DC amplifier (EPC-7, HEKA, Germany) by an Ag/AgCl electrode. The analog signal was digitized by the analog/digital board Digidata 1200 (Axon Instruments, PA) and transferred to a personal computer. Input/output curves (I/O) were obtained from two recordings at each of the following current intensities: 0, 20, 30, 40, 50, 100, 150, and 200 μA . Then, the stimulus intensity was adjusted to induce 60%–70% of the maximal response, a stable baseline was recorded for 20 min, and LTD was induced by a low-frequency stimulation (LFS) protocol (3,000 pulses at 5 Hz, LFS 5 Hz), as previously reported.¹⁴ The experimenter was blinded regarding the genetic condition of the animal.

Data analysis

Sweeps of 1,000 ms comprising the artifact, the non-synaptic components, and the fEPSP were acquired digitally using the software Axoscope 9.0 (Axon instruments, PA) and analyzed offline using the software Clampfit (Axon instru-

ments, PA); the peak amplitude values (mV) were exported in an Excel file. All data were plotted as mean values $\pm$ standard error of the mean (SEM), and statistical analysis was performed by using a one- or two-tailed paired or unpaired *t*-test, as appropriate. Differences were considered significant for $p < 0.01$. In LTD experiments, each amplitude value was normalized to the baseline (calculated as the mean of the fEPSP amplitudes recorded in the last 10 min before the beginning of the LFS 5 Hz). The effect of the LFS 5 Hz was evaluated 60 min after its application. Significant differences $\geq 10\%$ versus baseline were considered to be plastic changes of synaptic transmission, *i.e.*, LTD in case of depression at 60 min.

Results

Impairment in basal synaptic transmission

Figure 1A shows that the mean I/O value at the maximal stimulation intensity (200 μA) in basal synaptic transmission is significantly ($p = 0.004$) lower in Prh slices from 3-month-old Tg2576 mice ($1.09 \text{ mV} \pm 0.10 \text{ mV}$, $n = 8$ slices from 3 animals) than in those from control animals ($1.53 \text{ mV} \pm 0.09 \text{ mV}$; $n = 7$ slices from 2 animals).

Impairment in LFS 5 Hz-induced LTD

The LFS 5 Hz protocol induced LTD in Prh slices from 3-month-old control mice (baseline, $96.2\% \pm 1.2\%$; 60 min, $81.9\% \pm 4.0\%$; $p = 0.003$, $n = 5$ slices from 2 animals; Fig. 1B) but not in those from Tg2576 animals (baseline, $102.9\% \pm 2.3\%$; 60 min, $99.4\% \pm 3.0\%$; $p = 0.445$, $n = 8$ slices from 3 animals; Fig. 1C). The magnitude of LFS 5-Hz-induced LTD in control mice is consistent with previous observations in rats.^{12,14}

Discussion

Present data indicate that both basal synaptic transmission and LFS 5-Hz-induced LTD are impaired in Prh slices from 3-month-old Tg2576 mice. This result is particularly interesting because, to our knowledge, this is the first evidence of synaptic transmission and LTD impairment in the Prh in an animal model of AD and the earliest synaptic deficit in Tg2576 mice.

The impairment in LTD is consistent with visual recognition deficits observed in these mice at 5 months of age⁹ and in AD patients in the very early phase of the disease,¹³ as LTD in the Prh is believed to underlie this form of memory.^{11,12} On the other hand, the impairment in LTD may appear to be in contrast with previous reports in AD models showing enhancement of LTD and inhibition of LTP.^{15–19} However, a recent study has shown that A β -induced synaptic depression may employ signaling pathways of LTD to drive endocytosis of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) synaptic receptors and decrease in spine density, and this may result in a partial occlusion of LTD,¹⁷ as observed in our experiments.

Another interesting insight of present results is that synaptic transmission and LTD impairments occur at a very young (3 months) age. Previous studies in the same transgenic AD model have shown hippocampal synaptic dysfunction at 5 months but not at 2 months.⁸ Our observation can be explained by the very recent finding that Tg2576 mice show the highest levels of soluble A β oligomers in

FIG. 1. Basal synaptic transmission and low-frequency stimulation (LFS) 5-Hz-induced long-term depression (LTD). (A) Comparison of input/output (basal synaptic transmission) mean values obtained in perirhinal (Prh) slices from 3-month-old Tg2576 mice ($n=8$ from 3 animals) and from control animals ($n=7$ from 2 animals), showing a significant difference (* indicates $p < 0.01$) at $200 \mu\text{A}$. The application of LFS 5 Hz (3,000 pulses at 5 Hz) induced LTD in perirhinal cortex slices from littermate controls ($n=5$ from 2 animals; B), but not in those from Tg2576 mice ($n=8$ from 3 animals; C). fEPSP, Field excitatory postsynaptic potential.

hippocampus and associated structures at 3 months.⁷ Moreover, in another AD mice model bearing the Swedish mutation, the synaptophysin-immunoreactive presynaptic terminals in hippocampal CA1 were found to be 26%–32% lower than in controls at 2–3 months. Interestingly, a significant impairment in synaptic transmission was also observed at 1–4 months, whereas LTP was unaffected at 8–10 months.²⁰

In conclusion, these results suggest that synaptic transmission and LTD induction in the Prh are particularly sensitive to early AD pathogenic events, in accordance with early deficits in visual recognition memory in the same AD model⁹ and in AD patients.¹³ Further investigation is needed to characterize the mechanisms underlying the observed synaptic deficits. In particular, it would be interesting to investigate the role played by cholinergic neurotransmission, considering its importance in Prh synaptic plasticity and in visual recognition memory.¹¹

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Author Disclosure Statement

No competing financial interests exist.

References

- Wimo A, Prince M. Alzheimer's Disease International - World Alzheimer Report 2010 - The Global Economic Impact of Dementia. Available at www.alz.co.uk/research/world-report/. Accessed January 19, 2012.
- Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* 2002;297:353–356.
- Walsh DM, Selkoe DJ. Deciphering the molecular basis of memory failure in Alzheimer's disease. *Neuron* 2004;44:181–193.
- Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. *Lancet* 2006;368:387–403.
- Ash KH, Zahs KR. Probing the biology of Alzheimer's disease in mice. *Neuron* 2010;66:631–645.
- Mullan M, Crawford F, Axelman K, Houlden H, Lilius L, Winblad B, Lannfelt L. A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. *Nat Genet* 1992;1:345–347.
- Mustafiz T, Portelius E, Gustavsson MK, Hölttä M, Zetterberg H, Blennow K, Nordberg A, Unger Lithner C. Characterization of the brain β -amyloid isoform pattern at different ages of Tg2576 mice. *Neurodegener Dis* 2011;8:352–363.
- Jacobsen JS, Wu CC, Redwine JM, Comery TA, Arias R, Bowlby M, Martone R, Morrison JH, Pangalos MN, Reinhart PH, Bloom FE. Early-onset behavioral and synaptic deficits in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 2006;103:5161–5166.
- Taglialetela G, Hogan D, Zhang WR, Dineley KT. Intermediate- and long-term recognition memory deficits in Tg2576 mice are reversed with acute calcineurin inhibition. *Behav Brain Res* 2009;200:95–99.
- Brown MW, Aggleton JP. Recognition memory: What are the roles of the perirhinal cortex and hippocampus? *Nat Rev Neurosci* 2001;2:51–61.
- Warburton EC, Koder T, Cho K, Massey PV, Duguid G, Barker GR, Aggleton JP, Bashir ZI, Brown MW. Cholinergic neurotransmission is essential for perirhinal cortical plasticity and recognition memory. *Neuron* 2003;38:987–996.
- Massey PV, Phythian D, Narduzzo K, Warburton EC, Brown MW, Bashir ZI. Learning-specific changes in long-term depression in adult perirhinal cortex. *J Neurosci* 2008;28:7548–7554.
- Wolk DA, Signoff ED, Dekosky ST. Recollection and familiarity in amnesic mild cognitive impairment: A global decline in recognition memory. *Neuropsychologia* 2008;46:1965–1978.
- Aicardi G, Argilli E, Cappello S, Santi S, Riccio M, Thoenen H, Canossa M. Induction of long-term potentiation and depression is reflected by corresponding changes in secretion of endogenous brain-derived neurotrophic factor. *Proc Natl Acad Sci USA* 2004;101:15788–15792.
- Palop JJ, Mucke L. Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: From synapses toward neural networks. *Nat Neurosci* 2010;13:812–818.
- Kim JH, Anwyl R, Suh YH, Djamgoz MB, Rowan MJ. Use-dependent effects of amyloidogenic fragments of (beta)-amyloid precursor protein on synaptic plasticity in rat hippocampus in vivo. *J Neurosci* 2001;21:1327–1333.
- Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, Sisodia S, Malinow R. AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron* 2006;52:831–843.
- Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 2008;14:837–842.
- Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe DJ. Soluble oligomers of amyloid beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. *Neuron* 2009;62:788–801.
- Hsia AY, Masliah E, McConlogue L, Yu GQ, Tatsuno G, Hu K, Kholodenko D, Malenka RC, Nicoll RA, Mucke L. Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc Natl Acad Sci USA* 1999;96:3228–3233.

Address correspondence to:

Giorgio Aicardi
Department of Human and General Physiology
University of Bologna
Via San Donato 19/2
40127 Bologna
Italy

E-mail: giorgio.aicardi@unibo.it