Retention Period of Amyloid $\beta_{1-42}$ in the Brain Extracellular Fluid as the Toxicological Determinant in Freely Moving Rats

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The pathological significance of amyloid-$\beta_{1-42}$ ($\text{A}\beta_{1-42}$) dynamics is poorly understood in the brain extracellular compartment. Here we test which of the concentration or the retention is critical for $\text{A}\beta_{1-42}$ toxicity after injection of equal dose into dentate granule cell layer of freely moving rats. The toxicity of $\text{A}\beta_{1-42}$ (25 $\mu$M) was compared between injections at the rate of 0.25 $\mu$L/min for 4 min (fast injection) and 0.025 $\mu$L/min for 40 min (slow injection). Dentate gyrus long-term potentiation (LTP) was affected 1 and 2 h after the fast injection, but not 4 h. In contrast, LTP was affected even 72 h after the slow injection. $\text{A}\beta_{1-42}$ staining 5 min after finish of the slow injection was more intense in the dentate granule cell layer than of the fast injection. The present study indicates that the retention of $\text{A}\beta_{1-42}$ in the extracellular fluid is correlated with neuronal $\text{A}\beta_{1-42}$ uptake and plays a key role in $\text{A}\beta_{1-42}$ neurotoxicity. In the extracellular fluid of the dentate gyrus, the retention period of $\text{A}\beta_{1-42}$ is much more critical for $\text{A}\beta_{1-42}$ toxicity than $\text{A}\beta_{1-42}$ concentration. It is likely that $\text{A}\beta_{1-42}$ toxicity is accelerated by the disturbance of $\text{A}\beta_{1-42}$ metabolism in the dentate gyrus.

Key words amyloid-$\beta_{1-42}$; dentate gyrus long-term potentiation (LTP); $\text{Zn}^{2+}$; freely moving rat

INTRODUCTION

In familial Alzheimer’s disease (AD), more than 200 mutations have been identified in the three genes encoding the amyloid precursor protein (APP), presenilin 1 and presenilin 2 that are involved in amyloid-$\beta$ ($\text{A}\beta$) production. All the mutations increase $\text{A}\beta_{1-42}$ as a primary pathogenic agent in the AD development. Sporadic AD, the etiology of which is poorly understood, accounts for most AD patients. However, sporadic AD is essentially indistinguishable from familial AD in both pathological and neurological terms. Thus, it is important to understand why $\text{A}\beta$ accumulates in the sporadic AD brain and this understanding contributes to preventing the AD development.

Transsynaptic progression of $\text{A}\beta$-induced neuronal dysfunction is observed in the entorhinal-hippocampal network. The findings indicate that $\text{A}\beta$ release from the perforant pathway is closely linked with the damages of the postsynaptic dentate granule cells. On the other hand, Neprilysin is a rate-limiting peptidase involved in the physiological degradation of $\text{A}\beta$ in the brain and is most abundant in the stratum lacunosum-moleculare of the CA1–CA3 field and the molecular layer of the dentate gyrus in the mouse hippocampus. However, $\text{A}\beta_{1-42}$ dynamics is poorly understood in the extracellular compartment of the dentate gyrus. Here we test which of the concentration or the retention period in the extracellular compartment is critical for $\text{A}\beta_{1-42}$ neurotoxicity after injection of equal dose into dentate granule cell layer of freely moving rats.

MATERIALS AND METHODS

Animals and Chemicals Male Wistar rats (<20 weeks of age, Japan SLC, Hamamatsu, Japan) were caged under the standard conditions with a diurnal 12-h light cycle. The room temperature and relative humidity were controlled at 23 ± 1°C and 55 ± 5%, respectively. The rats were allowed free access to a standard laboratory food and water. The experiments were done in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka that refer to the American Association for Laboratory Animals Science and the guidelines laid down by the NIH in the U.S.A. (NIH Guide for the Care and Use of Laboratory Animals). This work has been approved by the Ethics Committee for Experimental Animals in the University of Shizuoka.

Synthetic human $\text{A}\beta_{1-42}$ (ChinaPeptides, Shanghai, China) was dissolved in saline before the experiments and used immediately.

In Vivo Long-Term Potentiation (LTP) Recording LTP was recorded at the perforant pathway-dentate granule cell synapses in freely moving and unanesthetized rats as reported previously. To monitor population spike (PS) amplitude, test stimuli (0.05Hz) were delivered at 20 s intervals and PS amplitudes were recorded for 10 min for the baseline recording in freely moving and unanesthetized rats. LTP was induced by delivering high-frequency stimulation (HFS; 10 trains of 20 pulses at 200Hz separated by 1 s) and under the same condition. PS amplitudes, which were measured for 10 min, were recorded 24–72 h (the baseline recording) and immediately (0 h) before high-frequency stimulation, and 1 h after HFS stimulation, averaged, and expressed as percentages of the mean PS amplitude recorded during the 10-min baseline period prior to LTP induction, which was expressed as 100%.

In Vivo $\text{A}\beta$ Immunostaining $\text{A}\beta$ immunostaining was done as reported previously. Saline (1 $\mu$L) and $\text{A}\beta$ in saline (25 $\mu$M, 1 $\mu$L) were bilaterally injected into the dentate granule cell layer of freely moving rats via injection cannulae at the rate of 0.25 $\mu$L/min for 4 min and the rate of 0.025 $\mu$L/min
for 40 min. Five minutes later, hippocampal slices were prepared, incubated at 4 °C with Aβ monoclonal antibody, 4G8 (COVANCE), and then incubated with Alexa Fluor 633 goat anti-mouse immunoglobulin G (IgG) secondary antibody. Images for immunostaining were captured using a confocal laser-scanning microscopic system and Alexa Fluor 633 fluorescence intensity was analyzed using NIH Image J to assess the level of Aβ uptake.

**Data Analysis** Data were expressed as means ± standard error. Differences among groups were analyzed by one-way ANOVA followed by post hoc test using the Tukey’s test for multiple comparisons (the statistical software, GraphPad...
A value of $p < 0.05$ was considered significant.

RESULTS

LTP is affected 1 h after the local injection of Aβ1-42 (25 μM, 1 μL) into the dentate gyrus of anesthetized rats at the rate of 0.25 μL/min for 4 min, consistent with memory deficit, but not after injection of Aβ1-42 (12.5 μM, 1 μL) at the rate of 0.25 μL/min for 4 min. In the present study, LTP was affected 1 h after injection of Aβ1-42 (25 μM, 1 μL) into the dentate gyrus of unanesthetized rats at the rate of 0.25 μL/min for 4 min (Fig. 1). LTP was also affected 2 h after the injection, but not 4 h.

In the case of injection at the slow rate of 0.025 μL/min for 40 min, LTP was affected 4 h after the injection and even 72 h (Fig. 2). As shown as the PS amplitudes just before LTP induction, the baseline of synaptic neurotransmission was not significantly modified after Aβ1-42 injection (Fig. 2), suggesting that the slow injection of Aβ1-42 has persistently toxic effect and acts on the mechanism of LTP induction. Aβ1-42 staining was more intensely observed in the dentate granule cell layer after the slow injection than the fast injection (Fig. 3).

DISCUSSION

The toxicity of Aβ1-42 (25 μM) was compared between injections at the rate of 0.25 μL/min for 4 min (fast injection) and 0.025 μL/min for 40 min (slow injection). LTP was affected 1 and 2 h after the fast injection, but not 4 h. In contrast, LTP was affected even 72 h after the slow injection. Aβ1-42 staining 5 min after finish of the slow injection was more intense in the dentate granule cell layer than of the fast injection. Aβ1-42 staining is observed around the nuclei of dentate granule cells in an extracellular Zn2+ dependent manner after the fast injection, suggesting that a portion of Zn-Aβ1-42 formed in the extracellular compartment is taken up into dentate granule cells in addition to the interaction with the plasma membranes.

The present study indicates that the retention of Aβ1-42 in the extracellular fluid is correlated with neuronal Aβ1-42 uptake and plays a key role in Aβ1-42 neurotoxicity. In the extracellular fluid of the dentate gyrus, the retention period of Aβ1-42 is more critical for Aβ1-42 toxicity than Aβ1-42 concentration.

Neuronal Aβ1-42 accumulation in the human brain occurs from approx. 40 years old and the Aβ1-42 level in normal brain are close to that of clinically AD-affected patients with progression of aging. Thus, aging is regarded as the most potent risk factor for sporadic AD. The levels of Neprilysin are characteristically reduced by 20–40% in the outer molecular layer and polymorphic layer of the dentate gyrus of aged mice, indicating that neprilysin is selectively decreased at the terminal zones and on axons of the lateral perforant pathway. It is likely that Aβ1-42 toxicity is accelerated by the disturbance of Aβ1-42 metabolism in the dentate gyrus.

Conflict of Interest The authors declare no conflict of interest.

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