Intraneuronally injected amyloid beta inhibits long-term potentiation in rat hippocampal slices

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Nomura I, Takechi H, Kato N. Intraneuronally injected amyloid beta inhibits long-term potentiation in rat hippocampal slices. J Neurophysiol 107: 2526–2531, 2012. First published February 15, 2012; doi:10.1152/jn.00589.2011.—Extracellular accumulation of amyloid beta (Aβ) is a hallmark of Alzheimer’s disease (AD). It has been reported that extracellular perfusion of Aβ inhibits long-term potentiation (LTP), which is strongly related to memory in animal models. However, it has recently been proposed that intracellular Aβ may be the first pathological change to occur in AD. Here, we have investigated the effect on LTP of intracellular injection of Aβ (Aβ1–40 or Aβ1–42) into hippocampal pyramidal cells using patch-clamp technique. We found that injection of 1 nM Aβ1–42 completely blocked LTP, and extracellular perfusion of a p38 MAPK inhibitor or a metabotropic glutamate receptor blocker reversed these blocking effects on LTP. Furthermore, we have examined the effects of different concentrations of Aβ1–40 and Aβ1–42 on LTP and showed that Aβ1–40 required a 1,000-fold higher concentration to attenuate LTP than 1 nM Aβ1–42. These results indicate that LTP is impaired by Aβ injected into genetically wild-type mice in the sliced hippocampus, suggesting an acute action of intracellular Aβ on the intracellular LTP-inducing machinery.

Alzheimer’s disease; p38 MAPK; metabotropic glutamate receptor; whole cell patch clamp

ALZHEIMER’S DISEASE (AD) is a neurodegenerative disorder characterized by progressive cognitive impairment. In AD pathology, beta-amyloid (Aβ), a peptide generated in the neuron and secreted extracellularly, is believed to be among the most pathological factors leading to neurodegeneration, membrane disruption, oxidative stress, impaired mitochondrial activity, synaptic dysfunction, and disturbance of axonal trafficking (Crouch et al. 2008). Extracellular deposition of Aβ fibrils and/or accumulation of Aβ oligomers are thought to cause neuronal degeneration (Small et al. 2001). However, recent findings have suggested that intraneuronal Aβ plays an important role in AD pathology (Li et al. 2007). Intracellular accumulation of Aβ has been detected prior to extracellular Aβ deposition in the brains of AD patients (Gouras et al. 2000), aging monkeys (Martin et al. 1994), and AD model mice (Chui et al. 1999; Oddo et al. 2003). Moreover, in triple transgenic AD model (3xTg) mice, which mimic many pathological factors leading to neurodegeneration, membrane disruption, oxidative stress, impaired mitochondrial activity, synaptic dysfunction, and disturbance of axonal trafficking (Crouch et al. 2008). Extracellular deposition of Aβ fibrils and/or accumulation of Aβ oligomers are thought to cause neuronal degeneration (Small et al. 2001). However, recent findings have suggested that intraneuronal Aβ plays an important role in AD pathology (Li et al. 2007). Intracellular accumulation of Aβ has been detected prior to extracellular Aβ deposition in the brains of AD patients (Gouras et al. 2000), aging monkeys (Martin et al. 1994), and AD model mice (Chui et al. 1999; Oddo et al. 2003). Moreover, in triple transgenic AD model (3xTg) mice, which mimic many critical hallmarks of AD, cognitive deficits were correlated with intraneuronal Aβ accumulation (Billing et al. 2005; Giménez-Llort et al. 2007). It has therefore been suggested that the intracellular accumulation of Aβ may be an early and critical event in neuronal dysfunction in AD (Glabe 2001; Gouras et al. 2005; Kienlen-Campard et al. 2002; LaFerla et al. 2007; Oddo et al. 2003; Tseng et al. 2004; Wirths et al. 2004; Zhang et al. 2002).

The relationship between extracellular Aβ and LTP has been extensively investigated. LTP attenuation has been observed in AD model mice (Jacobsen et al. 2006; Nalbantoglu et al. 1997). Our previous patch-clamp study has demonstrated that extracellular perfusion of a low concentration (500 nM) of Aβ to brain slices significantly impaired LTP in rat hippocampal CA1 pyramidal neurons (Nomura et al. 2005). This agrees with other studies showing that extracellular perfusion of soluble Aβ impairs LTP induction (Crouch et al. 2008; Selkoe 2008; Small et al. 2001). However, effects of intraneuronal Aβ on LTP induction are unknown.

The LTP impairment by extracellular Aβ is reported to be mediated by p38 mitogen-activated protein kinase (MAPK), c-Jun N terminal kinase, cyclin-dependent kinase 5 (Origlia et al. 2008; Saleshando and O’Connor 2000; Wang et al. 2004a, 2005), microglial activation (Wang et al. 2004b), tumor necrosis factor (Wang et al. 2005), or group I metabotropic glutamate receptor (mGluR) (Renner et al. 2010). It is also reported that the extracellular Aβ-mediating LTP impairment is recovered by nitric oxide donors (Puzzo et al. 2005), ubiquitin hydrolase (Gong et al. 2006), and insulin (Townsend et al. 2007). It is worth investigating involvement of such molecular signaling in intracellular Aβ-mediating LTP impairment if any exists. The present study first examined the effects of acute intraneuronal injection of Aβ on hippocampal LTP. Since LTP impairment was detected, we further studied its dependence on p38 MAPK and group I mGluR.

MATERIALS AND METHODS

Preparation of hippocampal slices. All experiments were performed in accordance with the guiding principles of the Physiological Society of Japan and with the approval of the Animal Care Committee of Kyoto University Graduate School of Medicine. Hippocampal slices (400 μm thick) were prepared from Wistar rats (17–21 days old) with a microslicer DTK-1000 (Dosaka, Kyoto, Japan). Slices were kept at room temperature for at least 60 min before experiments in normal medium composed of 124 NaCl, 3.0 KCl, 2.5 CaCl2, 1.25 MgCl2, 1.3 NaH2PO4, 26 NaHCO3, and 11 glucose (all in mM), bubbled with a mixture of 95% O2 and 5% CO2.

Electrophysiology. The whole cell voltage-clamp recordings were from the soma of pyramidal neurons located in CA1 of the hippocampus. Pyramidal neurons were identified by the typical morphological shape under the microscope and also by the electrophysiological responses. A patch pipette (5–7 MΩ) was filled up with the internal solution containing 120 CsMeSO4, 5 CsCl, 2 MgCl2, 10 HEPES, 0.2

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EGTA, 2 Na2ATP, 0.4 GTP, 5 NaCl, and 5 QX-314 (in mM). The pH was adjusted to 7.3 with CsOH, and osmolality was 290 mOsm. In all experiments, bicuculline (10 μM) and CGP55854 (1 μM) were added to block actions of GABA receptors. The EPSC-9 patch-clamp amplifier and program package PULSE-PULSEFIT (HEKA Electronics, Lambrecht, Germany) were used for data acquisition. Series resistance (SR) was continuously recorded and monitored between 15 and 35 MΩ. Data were excluded if changes of SR exceeded 20% during one whole experiment. The holding potential was kept at −70 mV. Excitatory postsynaptic current (EPSC) was evoked using a stimulating glass capillary microelectrode filled with normal medium and placed in the stratum radiatum. EPSCs were generated synchronously by injecting current into the pyramidal neuron. The magnitude of LTP was determined by expressing the EPSC amplitude averaged over the 25–30 min period immediately prior to TBS. For LTP induction, theta burst stimulation (TBS) was used. Stable baseline recordings were obtained for more than 5 min prior to TBS. Ten series of theta bursts was applied ~20 min after whole cell break-in to allow sufficient Aβ infusion. TBS consists of four pulses of 100-Hz current. The duration of each burst is 200 ms. Coincident with each burst, postsynaptic depolarization at 0 mV was generated synchronously by injecting current into the pyramidal neuron. The magnitude of LTP was determined by expressing the EPSC amplitude averaged over 25–30 min post-TBS as percent of the baseline EPSC amplitude averaged over the 5-min period immediately prior to TBS.

Drug application and experimental design. Three different species of Aβ peptides (Aβ1–42, Aβ1–40, Aβ42; Peptide Institute, Osaka, Japan) were dissolved in DMSO and diluted to desired final concentrations in the internal solution just before starting experiments. Each Aβ peptide in the internal solution was infused by diffusion into the cytoplasm of a pyramidal cell through the recording patch pipette. Internal solution that contains 0.1% DMSO, but not Aβ peptides, was used for some control experiments, which are referred to as the no-Aβ controls or group.

To examine further mechanisms of LTP inhibition by intracellular Aβ1–42 (1 nM), cell-permeable drugs were administrated. Hippocampal slices were preincubated with medium containing SB203580 (1 μM), a p38 MAPK inhibitor, or MPEP (5 μM), a group I metabotropic glutamate receptor (mGluR) antagonist, for at least 40 min prior to injection of Aβ1–42. This was to ensure that the drugs penetrate slices into the depth. All the drugs were purchased from Nacalai (Kyoto, Japan) unless otherwise noted.

Gel electrophoresis. The aggregation state of Aβ1–42 in the pipette solution was assessed by SDS-PAGE. Samples were taken from each Aβ dissolved in pipette solution and added to SDS loading buffer (50 mM Tris/HCl, pH 6.8, 4% SDS, 1.0 mg/ml bromophenol blue, 20% glycerol, 10% 2-mercaptoethanol). The mixture was separated by tricine-SDS gel electrophoresis (16.5% polyacrylamide) and visualized by silver staining (PlusOne Silver Staining Kit, Protein, GE Healthcare Bio-Sciences, Tokyo, Japan).

Statistical analysis. Data are presented as means ± SE. For statistics, Student’s t-test and one- or two-way ANOVA followed by Dunnett’s test were used (SPSS v18, Japan IBM, Tokyo, Japan). The significance level was set at P < 0.05.

RESULTS

Low concentrations of intracellular Aβ do not affect basal synaptic transmission. We first examined the effect of intracellular Aβ1–42 perfusion on basal synaptic transmission at Schaffer collateral-CA1 pyramid cell synapses. EPSCs were evoked at 0.05 Hz for 60 min, without any attempt to induce LTP. With Aβ1–42 intracellularly infused at 1 μM (Fig. 1, B and C) or 1 nM (Fig. 1C), the peak amplitude of the EPSC remained unchanged. The averaged amplitudes of EPSCs over 25–30 min in the time scale (Fig. 1C) were 97 ± 6% of the

Fig. 1. Basal synaptic transmission was not affected by intracellular injection of Aβ1–42. A and B: representative recordings obtained from a neuron without Aβ injected (A; no-Aβ) and another neuron with Aβ1–42 injected (1 μM) (B; Aβ42). Series resistance (SR) is shown at the bottom of each recording. Each point represents the mean amplitude of 3 excitatory postsynaptic currents (EPSCs). Insets: typical traces of recordings at the times indicated. Scale bars, 50 pA, 50 ms. C: averaged EPSC amplitudes (means ± SE) for the no-Aβ control and Aβ1–42 (1 nM) and Aβ1–42 (1 μM) groups. Averaged amplitudes were based on EPSCs recorded at 25–30 min in the time scale, and expressed as percent of the baseline EPSC amplitudes averaged over the 5-min period from −6 to −1 min. Recordings at time 0 are lacking because it was intended that the recording condition should be the same in these baseline recordings and the LTP experiments (see Fig. 2). For LTP induction, EPSC recording was interrupted at time 0, and instead the burst protocol was applied. D: molecular composition of Aβ1–42 in the pipette solution. Aβ1–42 was taken from the pipette solution and prepared for SDS-PAGE. Silver-stained bands are positive at the trimer and tetramer positions, although much less extensive than the monomer band.
EPSC size averaged over the 5-min period from -6 to -1 min in the no-\(\alpha\beta\) control (\(n = 22\)), 95 ± 9% in the 1 \(\mu\)M group (\(n = 11\)), and 101 ± 8% in the 1 nM group (\(n = 7\)). These were not significantly different \([F(2,37) = 0.077; 1\)-way ANOVA\]. It appears that the EPSC shape also remained unchanged (Fig. 1B, insets, for the 1-\(\mu\)M group) compared with the no-\(\alpha\beta\) control group (Fig. 1A, insets).

The molecular composition of \(\alpha\beta_{1–42}\) contained in the internal solution was briefly analyzed by SDS-PAGE (Fig. 1D). The vast majority of the injected \(\alpha\beta_{1–42}\) was in the form of monomer, while trimers and tetramers were also included at much lower concentrations.

Intracellular perfusion of \(\alpha\beta_{1–42}\) (1 nM) impairs LTP induction. We then examined the effects of intracellular \(\alpha\beta_{1–42}\) (1 nM) on LTP induction. TBS successfully evoked LTP in no-\(\alpha\beta\) control neurons (Fig. 2, A and C, no-\(\alpha\beta\)), whereas intracellular injection of 1 nM \(\alpha\beta_{1–42}\) blocked LTP induction (Fig. 2, B and C, \(\alpha\beta\)). The amplitude of EPSC at 30 min post-TBS, expressed as percent of the baseline amplitude, was significantly smaller in the \(\alpha\beta_{1–42}\) group (100 ± 10%, \(n = 11\); \(t\)-test, \(P < 0.05\)) than in the no-\(\alpha\beta\) control group (162 ± 12%, \(n = 14\), Fig. 2C). In the no-\(\alpha\beta\) control experiments, no peptide was intracellularly injected. For a more adequate control, we intraneuronally injected \(\alpha\beta_{22–1}\), the reverse peptide (R-\(\alpha\beta\)) that is assembled from the same ingredient amino acids as \(\alpha\beta_{1–42}\) in the reverse direction. Injection of \(\alpha\beta_{22–1}\) (1 nM) did not block LTP induction (Fig. 2C, R-\(\alpha\beta\); 186 ± 24%, \(n = 7\)), which indicates at least qualitatively that the \(\alpha\beta_{1–42}\)-induced LTP blockade is specifically attributed to \(\alpha\beta_{1–42}\) but not to injection of any peptide of a similar molecular weight.

\(SB203580\) and MPEP reverse LTP inhibition by intracellular \(\alpha\beta_{1–42}\). It is reported that LTP impairment induced by extracellular \(\alpha\beta\) depends on p38 MAPK and group I mGluRs (Saleshando et al. 2000; Wang et al. 2004a, 2005). To examine if this is also the case for LTP impairment induced by intracellularly injected \(\alpha\beta_{1–42}\), we administrated the p38 MAPK inhibitor \(SB203580\) or the group I mGluR antagonist MPEP in addition to intracellularly injected \(\alpha\beta_{1–42}\). With \(SB203580\) and MPEP applied, the magnitudes of LTP were 168 ± 29% (Fig. 2D, \(n = 8\)) and 164 ± 33% (Fig. 2E, \(n = 9\)), respectively.

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Fig. 2. Intracellularly injected \(\alpha\beta_{1–42}\) (1 nM) inhibits LTP, depending on p38 MAPK and group I mGluRs. A and B: representative EPSC recordings obtained from a neuron without \(\alpha\beta\) injected (A; no-\(\alpha\beta\)) and another neuron with \(\alpha\beta_{1–42}\) injected (1 nM) (B; \(\alpha\beta\)). In this figure, \(\alpha\beta\) refers to \(\alpha\beta_{1–42}\), and its concentration was always 1 nM. TBS was delivered at time 0 (arrows). After TBS, the EPSC amplitude was enhanced in the no-\(\alpha\beta\) group but not in the \(\alpha\beta\) group. Scale bars, 50 pA, 50 ms. C: averaged data for the no-\(\alpha\beta\), \(\alpha\beta\), and R-\(\alpha\beta\) groups. R-\(\alpha\beta\) refers to \(\alpha\beta_{22–1}\) (1 nM). D and E: averaged EPSC amplitudes for neurons administered intracellularly with \(\alpha\beta\) and extracellularly with \(SB203580\) (D) or MPEP (E). TBS was delivered at time 0 (arrows). F: comparison of the LTP magnitude among the different experimental groups. Among the 4 groups of neurons, into which \(\alpha\beta\) or R-\(\alpha\beta\) was intracellularly injected, the LTP magnitude differs significantly, and the data for the \(\alpha\beta\) group is significantly smaller than those for any other group (1-way ANOVA followed by Dunnett’s test, \(^*P < 0.03\)).
We then performed one-way ANOVA over the four experimental groups that were subjected to injection of $\alpha\beta_{1-42}$ or $\alpha\beta_{42-1}$ (R-$\alpha\beta$, $\beta\alpha$, $\beta\alpha + \alpha\beta$, and $\beta\alpha + \alpha\beta$; Fig. 2F). An across-group difference in LTP magnitude was detected [$F_{2,31} = 2.96, P < 0.05$], and, subsequently, Dunnett’s test revealed that the LTP magnitude is significantly larger in the $\alpha\beta_{1-42}$ group (100 ± 10%, $n = 11, P < 0.03$) than in the other three groups. Finally, it was confirmed that LTP was normally induced with SB203580 (152 ± 19%, $n = 8$) or MPEP alone without $\alpha\beta_{1-42}$ injected (138 ± 30%, $n = 5$).

**LTP inhibition shows an inverse concentration-response curve for intracellular $\alpha\beta_{1-42}$.** In our previous study, we found that extracellular perfusion with $\alpha\beta_{1-42}$ impaired induction of LTP in a dose-dependent manner (Nomura et al. 2005). Thus, we attempted to clarify the effects of other concentrations of $\alpha\beta_{1-42}$ on induction of LTP in the same way. Despite our expectation that higher concentrations of $\alpha\beta_{1-40}$ may inhibit LTP induction, the stronger, higher concentrations of $\alpha\beta_{1-42}$ failed to inhibit LTP (1 μM, 155 ± 17%, $n = 10$; 100 nM, 129 ± 16%, $n = 10$; 10 nM, 119 ± 16%, $n = 11$; not significantly different than the no-$\alpha\beta$ control; Fig. 3A). We also examined the effects of intracellularly injected $\alpha\beta_{1-40}$ on LTP magnitude. Intracellular perfusion of $\alpha\beta_{1-40}$ (1 μM) significantly impaired LTP induction, since the LTP magnitude (106 ± 16%, $n = 7$) was not significantly different from the no-$\alpha\beta$ control data. However, 100 nM and lower concentrations of $\alpha\beta_{1-40}$ failed to inhibit LTP induction (Fig. 3A). These results strongly suggest different dose dependencies of $\alpha\beta_{1-40}$ and $\alpha\beta_{1-42}$ effects on LTP induction. Two-way ANOVA revealed a significant interaction between the concentration effect and the $\alpha\beta$ species effect ($P < 0.01$), which confirmed that the dose-dependent curves of the two $\alpha\beta$ species significantly differ. This agrees with the finding that intracellular perfusion of $\alpha\beta_{1-40}$ (1 μM) impaired LTP induction (106 ± 16%, $n = 7$, Fig. 3B), whereas $\alpha\beta_{1-42}$ (1 μM) left it intact (155 ± 17%, $n = 10$). The LTP magnitudes in neurons injected with $\alpha\beta_{1-40}$ at the other concentrations were 139 ± 25% ($n = 7$, 100 nM), 131 ± 17% ($n = 6$, 10 nM), and 166 ± 13% ($n = 5$, 1 nM), and were not significantly different from the no-$\alpha\beta$ control group.

**DISCUSSION**

The present report is, at least to our knowledge, the first demonstration of impaired induction of LTP following intracellular perfusion with a low concentration of $\alpha\beta_{1-42}$ without effects on basal synaptic transmission. Oddo et al. (2003) showed LTP impairment at the stage of intraneuronal accumulation of $\alpha\beta_{1-42}$ in the 3xTg-AD model mouse. The presence of intracellular $\alpha\beta_{1-42}$ has been demonstrated by other groups as well (LaFerla et al. 2007, for review). This intracellular accumulation of $\alpha\beta_{1-42}$ in 3xTg mice has failed to be corroborated in a recent study (Winton et al. 2011) in which 3xTg mice were rendered genetically deficient for $\beta$-secretase, the enzymatic activity required for $\alpha\beta_{1-42}$ generation from the precursor protein. A later examination (Wirths et al. 2012) did not confirm a general deficiency of intraneuronal $\alpha\beta_{1-42}$ in the forebrain of 3xTg mice, but nevertheless concluded that only minor levels of intraneuronal $\alpha\beta_{1-42}$ was detected at least in the CA1 region. The possibility therefore arises that LTP impairment in 3xTg mice might be attributable to factors other than intracellular $\alpha\beta_{1-42}$ accumulation. In the present experiments, it is clearly shown that intracellular injection of $\alpha\beta_{1-42}$ causes LTP suppression in wild-type rat neurons, although evaluation of the role of intraneuronal $\alpha\beta_{1-42}$ in LTP impairment in 3xTg mice is simply beyond the scope of the present study.

Low concentrations of extracellular $\alpha\beta_{1-42}$ have been well known to suppress hippocampal LTP (Ma et al. 2011; Shankar et al. 2008; Walsh et al. 2002). It is reported that extracellular $\alpha\beta_{1-42}$ binds directly to nicotinic acetylcholine receptors (Wang et al. 2000), modulates glutamate receptor trafficking (Hsieh et al. 2006; Kamenetz et al. 2003; Snyder et al. 2005), and affects cellular prion protein functioning (Laurén et al. 2009). Prion protein is further shown to mediate the LTP-suppressing effect of extracellular $\alpha\beta_{1-42}$ (Barry et al. 2011; Laurén et al. 2009), although the issue of $\alpha\beta_{1-42}$ binding to surface receptors in general appears to be in dispute (e.g., Benilova and De Strooper 2010). Along with such proposed interactions of $\alpha\beta_{1-42}$ with membrane proteins, a direct internalization of $\alpha\beta_{1-42}$ may still be possible. In fact, receptors for advanced glycation end product (RAGE) has been reported to internalize extracellular $\alpha\beta_{1-42}$, which may substantiate membrane transport of $\alpha\beta_{1-42}$ from the extracellular to the intracellular space (Deane et al. 2003; Origlia et al. 2008). Such a transmembrane transport may explain a marked difference in the $\alpha\beta_{1-42}$ concentration required for LTP suppression in our...
present and previous findings. In the present experiments, 1 nM intracellular Aβ1-42 is sufficient to inhibit induction of LTP, as opposed to the requirement of 200 nM concentration of extracellular Aβ1-42 in our previous experiments done with the same protocol (Nomura et al. 2005). The much lower concentration requirement for intracellular Aβ1-42 appears to be consistent with the idea that extracellularly applied Aβ1-42 may be transported into the intracellular space, which offers the site of Aβ1-42 action. However, this interpretation may be inconsistent with the previous report (Walsh et al. 2002), which showed that the extracellular Aβ1-42 concentration required for suppressing LTP is much lower than in our previous study (Nomura et al. 2005). Also, Walsh et al. (2002) suggested that the transport of Aβ1-42 from the intra- to extracellular compartment, i.e., in the direction opposite to our proposal, is responsible for LTP suppression.

The impairment of LTP induced by extracellular perfusion of Aβ1-42 has been reported to depend on p38 MAPK and group I mGluRs (Origlia et al. 2008; Saleshando et al. 2000; Wang et al. 2004a, 2005). The present experiments have confirmed that LTP suppression by intracellular Aβ1-42 also depends on the lines of signaling represented by these two molecules (Fig. 2, D-E, F). Given the intracellular localization of p38 MAPK, its interaction with intracellular Aβ1-42 may be transported into the intracellular space, which offers the site of Aβ1-42 action. However, this interpretation may be inconsistent with the previous report (Walsh et al. 2002), which showed that extracellular Aβ1-42 concentration required for suppressing LTP is much lower than in our previous study (Nomura et al. 2005). Also, Walsh et al. (2002) suggested that the transport of Aβ1-42 from the intra- to extracellular compartment, i.e., in the direction opposite to our proposal, is responsible for LTP suppression.

In our experiments, the higher concentrations of intracellular Aβ1-42 (1 μM and 100 nM) failed to impair LTP induction (Fig. 3A). Although we have no clear explanation for this dose dependence, the following interpretation might be possible. At high concentrations, Aβ1-42 may form aggregates that could be less toxic or less mobile than unaggregated Aβ1-42 at 1 nM. Such aggregates may take a form of fibrils, which have been generally known to be less toxic than oligomers. Such aggregation specific to high concentrations of Aβ1-42 could be a possible reason for the lack of Aβ1-42 effects at relatively high concentrations in the present experiments.

REFERENCES


