Blockade of Long-Term Potentiation by β-Amyloid Peptides in the CA1 Region of the Rat Hippocampus In Vivo

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Freir, Darragh B., Christian Holscher, and Caroline E. Herron. Blockade of long-term potentiation by β-amyloid peptides in the CA1 region of the rat hippocampus in vivo. J Neurophysiol 85: 708–713, 2001. The effect of intracerebroventricular (icv) injections of β-amyloid peptide fragments Aβ[15–25], Aβ[25–35], and Aβ[35–25] were examined on synaptic transmission and long-term potentiation (LTP) in the hippocampal CA1 region in vivo. Rats were anesthetized using urethane, and changes in synaptic efficacy were determined from the slope of the excitatory postsynaptic potential (EPSP). Baseline synaptic responses were monitored for 30 min prior to icv injection of Aβ peptides or vehicle. High-frequency stimulation (HFS) to induce LTP was applied to the Schaffer-collateral pathway 5 min or 1 h following the icv injection. HFS comprised 3 episodes of 10 stimuli at 200 Hz, 10 times, applied at 30-s intervals. Normal LTP measured 30 min following HFS, was produced following icv injection of vehicle (191 ± 17%, mean ± SE, n = 6) or Aβ[15–25; 100 nmol] (177 ± 6%, n = 6) 1 h prior to HFS. LTP was, however, markedly reduced by Aβ[25–35; 10 nmol] (129 ± 9%, n = 6, P < 0.001) and blocked by Aβ[25–35; 100 nmol] (99 ± 6%, n = 6, P < 0.001). Injection of the reverse peptide, Aβ[35–25], also impaired LTP at concentrations of 10 nmol (136 ± 3%, n = 6, P < 0.001) and 100 nmol (144 ± 7, n = 8, P < 0.05). Using a different protocol, HFS was delivered 5 min following Aβ injections, and LTP was measured 1 h post HFS. Stable LTP was produced in the control group (188 ± 15%, n = 7) and blocked by Aβ[25–35; 100 nmol] (108 ± 15%, n = 6, P < 0.001). A lower dose of Aβ[25–35; 10 nmol] did not significantly impair LTP (176 ± 30%, n = 4). The Aβ-peptides tested were also shown to have no significant effect on paired pulse facilitation (interstimulus interval of 50 ms), suggesting that neither presynaptic transmitter release or activity of interneurons in vivo are affected. The effects of Aβ on LTP are therefore likely to be mediated via a postsynaptic mechanism. This in vivo model of LTP is extremely sensitive to Aβ-peptides that can impair LTP in a time- ([25–35]) and concentration-dependent manner ([25–35] and [35–25]). These effects of Aβ-peptides may then contribute to the cognitive deficits associated with Alzheimer’s disease.

INTRODUCTION

Alzheimer’s disease (AD) is a neurodegenerative disorder leading to loss of memory, progressive decline in cognitive function, and premature death. One of the hallmarks of AD is the presence of numerous senile plaques and neurofibrillary tangles in the affected brain regions. The main constituent of these plaques is β-amyloid peptide (Aβ), a 39–42 amino acid peptide derived by proteolysis of amyloid precursor protein (APP). There appears to be a correlation between amyloid load in the cortex and disease progression on a cognitive level (Braak and Braak 1997). It is generally accepted that Aβ is involved in neuronal degeneration; however, the mechanism of action and how it causes dementia have yet to be determined.

It has been reported previously that the synthetic Aβ-peptide [22–35] and [25–35] produced neurotoxic effects in cell culture studies (Gray and Patel 1995; Takadera et al. 1993). The proposed mechanisms suggested include increases in reactive oxygen species (Behl et al. 1994) and increased intracellular calcium levels (Mattson et al. 1992). In vivo injection of both Aβ[25–35] and Aβ[1–40] produces neurodegeneration and neurite dystrophy similar to that seen in autopsy samples of AD patients (Pike et al. 1991, 1992). APP is expressed and localized on glutamatergic neurons (Ouimet et al. 1994), while cellular damage in the brains of patients with Alzheimer’s disease is found predominantly in areas that display glutamatergic synaptic plasticity in the adult brain (Arendt et al. 1998). Prolonged infusion of synthetic Aβ into the brain can produce learning and memory deficits in rats (Nitta et al. 1997; Sweeney et al. 1997), while overexpression of Aβ in transgenic mice is also associated with cognitive decline (Chapman et al. 1999; Nalbantoglu et al. 1997). Aβ neurotoxicity has also been shown to be independent of plaque formation (Mucke et al. 2000). Chronic infusion of low doses of Aβ[1–40] have also been shown to reduce long-term potentiation (LTP) in vivo (Itoh et al. 1999).

Hippocampal LTP is an activity-dependent increase in the synaptic response that is used as a cellular model of learning and memory. It is dependent on Ca2+ influx and subsequent activation of calcium-dependent second-messenger processes including protein synthesis and phosphorylation. Experiments using transgenic mouse models and gene targeting have shown a close association between impaired hippocampal LTP and behavioral learning and memory deficits. LTP may then represent a good model with which to examine the neuronal mechanisms involved in diseases associated with cognitive decline. In the CA1 region, LTP requires activation of N-methyl-d-aspartate (NMDA)–type glutamate receptors (Collingridge et al. 1983) and/or L-type voltage-gated calcium channels (Grover and Teyler 1990; Morgan and Teyler 1999). It has been used as a model of activity-dependent synaptic plasticity that may underlie some forms of learning and memory. Previous reports have indicated that β-amyloid peptide

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can alter the activation kinetics of L-type calcium channels (Ekincci et al. 1999; Ueda et al. 1997) in addition to increasing NMDA receptor–mediated synaptic responses (Wu et al. 1995b). We decided therefore to examine the effects of Aβ-peptides on hippocampal LTP and synaptic transmission. In this report we document our investigation of the effects of intracerebroventricular (icv) injections of several Aβ-peptides on synaptic transmission and LTP in the CA1 region of the rat hippocampus in vivo. The peptides examined included Aβ[15–25], which is part of the extracellular hydrophilic domain and does not contain the active sequence of Aβ (Pike et al. 1995), as such Aβ[15–25] was used as a control peptide. Aβ [25–35] is the most neurotoxic fragment (Yankner et al. 1990) that comprises part of the membrane spanning region and includes a hydrophobic domain. Finally, Aβ [35–25] was used to examine the effects of reversing the sequence of the active region to determine whether biological activity of the peptide was affected.

M E T H O D S

Male Wistar rats (150 –200 g) were surgically prepared for acute recordings. Rats were anesthetized with 1.5 g/kg urethan (ethyl carbamate) and placed in a stereotaxic device for surgery and recording. Heating pads (Braintree Scientific) were used to maintain the temperature of the animals at 37.0 ± 0.5°C. Small holes were drilled in the skull at the sites of the reference, recording, and stimulating electrodes. An additional hole was drilled to introduce a cannula for icv injection of drugs/vehicle. The recording electrode was positioned in the stratum radiatum area of CA1 (3 mm posterior to bregma, 2 mm lateral to bregma). A bipolar stimulating electrode was placed in the Schaffer collateral/commissural pathway, distal to the recording electrode (4 mm posterior, 3 mm lateral). Physiological (Leung 1980) and stereotaxic indicators were used to lower electrodes slowly through the cortex into the hippocampus. Test stimuli were delivered to the Schaffer-collateral commissural pathway every 30 s. Electrodes were positioned to record a maximal evoked field excitatory postsynaptic potential (EPSP). Baseline EPSPs were set within the range 0.8 –1 mV, which corresponded to 40% of the maximal response. Rats were injected icv with Aβ-peptide fragments (100 or 10 nmol in 5 μL) or vehicle (distilled water; 5 μL). In each protocol used, LTP was induced by applying high-frequency stimuli (HFS; 3 trains of 10 stimuli at 200 Hz) at a stimulus intensity that evoked a field EPSP of approximately 1.5–2.0 mV, approximately 80% of maximal EPSP. Stimulating (bi-polar stainless steel; 0.125 mm diam) and recording (mono-polar stainless steel; 0.125 mm diam) electrodes were obtained from Plastics One. Peptides were obtained from Bachem (Saffron Walden, UK).

Data analysis

Extracellular field potentials were amplified (×100), filtered at 5 kHz, digitized, and recorded using Mac Lab software acquisition system. The EPSP slope was used to measure synaptic efficacy. EPSPs are expressed as a percentage of the mean initial slope measured during the first 10 min of the baseline-recording period. Data were analyzed using ANOVA measured over 5 min prior to LTP induction and either at 25–30 or 55–60 min following the induction of LTP. The Tukey-Kramer multiple comparisons test was used to determine statistical significance.

Stimulation protocol

Field EPSPs were evoked in the CA1 region using low-frequency stimulation (0.033 Hz) to obtain at least 30 min of stable baseline prior to icv injection of Aβ-peptide or vehicle. At a time of either 5 min or 1 h following the icv injection, a series of high-frequency stimuli were delivered to induce potentiation of the synaptic response. Low-frequency stimulation was then used to evoke EPSPs for a further period of 30 min to 1 h to monitor any change in the synaptic response. Paired-pulse facilitation (PPF) with an inter-stimulus interval of 50 ms was also examined before icv injection of Aβ-peptides and again 90 min following icv injection (30 min following induction of LTP).

R E S U L T S

In control experiments, icv administration of vehicle had no effect on baseline synaptic transmission, or PPF monitored for up to 1 h following injection. When HFS was applied 1 h following vehicle injection, LTP measured 191 ± 17% (n = 6, mean ± SE, P < 0.05, ANOVA, 30 min following HFS; Fig. 1). LTP of a similar magnitude was also produced following icv injection of Aβ-peptide and again 90 min following icv injection (30 min following induction of LTP).

The effects of Aβ[15–25], Aβ[25–35], and the reverse peptide Aβ[35–25] were examined. Baseline synaptic transmis-

![Effect of icv injection of 100 nmol Aβ[15–25] on normalized low-frequency synaptic transmission 1 h prior to the induction of long-term potentiation (LTP). Aβ[15–25] does not contain the neurotoxic sequence of Aβ peptide. Averaged (n = 3) field excitatory postsynaptic potentials (EPSPs) are shown 5–10 min pretetanus and 30 min following tetanus for control (vehicle; 1, 2) and Aβ[15–25] (100 nmol; 3, 4) injected rats. Calibration bars: 1 mV; 5 ms. Injection of Aβ[15–25] (100 nmol; 3) does not affect low-frequency (0.033 Hz) synaptic transmission compared with control (1) monitored for 1 h following injection. LTP was measured 30 min following a 200-Hz tetanus with an increase in the EPSP slope of 191 ± 17% (n = 7) for control (vehicle) and 177 ± 6% (n = 6) for Aβ[15–25] (100 nmol).]
sion was monitored for periods of up to 1 h following icv injection of Aβ-peptides. There was found to be no significant effect on baseline EPSPs evoked at low frequency (0.033 Hz, Figs. 1, 3, and 4) or on PPF (Table 1).

Injection of Aβ[15–25], 1 h before the application of HFS had no significant effect on LTP (177 ± 6%, n = 6) compared with the control group (191 ± 17%, n = 6; Fig. 1). Aβ[15–25] does not incorporate the trans-membrane, neurotoxic sequence of Aβ and was a good indication that injection of peptide was not causing a nonspecific effect on synaptic transmission and plasticity.

Injection of the active neurotoxic portion of Aβ, namely Aβ[25–35] (Yankner et al. 1990), however, was found to significantly impair LTP in a time- and concentration-dependent manner. When 10 nmol Aβ[25–35] was injected 5 min prior to HFS, there was no effect on LTP (176 ± 30%, n = 4) compared with control (188 ± 15%; n = 7), while 100 nmol was found to reduce significantly LTP (108 ± 15%, n = 6, P < 0.001; Fig. 2). To examine the effect of prolonging the application of Aβ[25–35], icv injections were performed 1 h prior to the induction of LTP. Under these conditions, the lower 10 nmol concentration of Aβ[25–35] was now found to reduce significantly LTP (129 ± 9%, n = 6, P < 0.01) when compared with the control group (191 ± 17%; n = 6), while 100 nmol blocked LTP (99 ± 6%, n = 6, P < 0.001; Fig. 3). This indicates that even short-term exposure to a dose of 100 nmol/5 μL of Aβ[25–35] can impair LTP, although baseline synaptic transmission remains unaffected.

The effect of reversing the sequence of the neurotoxic Aβ fragment on LTP and baseline synaptic transmission was also examined. When Aβ[35–25] 10 and 100 nmol was injected 1 h prior to HFS, there was a significant reduction in LTP (136 ± 3%, n = 6, P = 0.01) and (144 ± 7%, n = 8, P < 0.05) compared with control (191 ± 17%, n = 6; Fig. 4).

PPF, induced by applying two stimuli at an inter-stimulus interval of 50 ms was recorded immediately prior to injection of Aβ fragments/vehicle and again 30 min following HFS. The PPF ratio was measured as the slope of EPSP2/EPSP1 (Table 1). None of the Aβ peptides tested had a significant effect on PPF (paired t-test), although there was a trend toward a decrease in facilitation.

**DISCUSSION**

In this study we have shown for the first time in vivo that the impairment of LTP by Aβ[25–35] peptide is both time and concentration dependent. This is demonstrated by the greater decrease in LTP observed when tetanic stimulation was given 1 h and not 5 min post-injection. Also, 100 nmol Aβ[25–35] had a more powerful blocking effect than 10 nmol Aβ[25–35]. LTP was impaired by the most neurotoxic fragment, Aβ[25–35] (Yankner et al. 1990), containing the lipophilic portion of Aβ (amino acids 28–35). Interestingly, Aβ[35–25], the “reverse” sequence peptide, previously reported to have no effect on LTP in vitro (Chen et al. 2000), caused a marked reduction in plasticity at both 10 and 100 nmol, while Aβ[15–25] had no effect on LTP.

What are the potential mechanisms involved in the effects of Aβ-peptides on LTP? There are two types of channel known to be implicated in the induction of LTP that may be disrupted by active Aβ-peptide: namely the L-type voltage-gated calcium channel, activated by the high stimulus frequencies of 200 Hz used in our study to induce LTP (Grover and Teyler 1990; Morgan and Teyler 1999) and/or the NMDA receptor/channel complex (Collingridge et al. 1983). Alteration of the activation
kinetics of either of these channels by Aβ could influence LTP induction.

Aβ can cause the formation of calcium-permeable pores in cell membranes (Engström et al. 1995) and block the fast inactivating potassium channel (Good and Murphy 1996); both mechanisms would produce an increase in intracellular calcium. In addition, the L-type calcium channel current is increased by Aβ (Ueda et al. 1997) acting via mitogen-activated protein (MAP) kinase (Ekinci et al. 1999). The disruption of intracellular calcium homeostasis is likely to play a major role in LTP impairment. Aβ[1–40] has been reported to cause a reduction in LTP in vivo (Cullen et al. 1997), yet can also produce a potentiation of LTP in vitro, possibly due to an increase in activation of the NMDA receptor (Wu et al. 1995a,b). An increase in NMDA receptor function has also been reported in hippocampal slices from transgenic mice over expressing human APP (Hsiao et al. 1999). Depending on the degree of activation of the NMDA receptor, and consequent calcium influx, it is possible to induce LTP or long-term depression (Mulkey and Malenka 1992). Prolonged low level activation of the NMDA receptor/channel can also reduce the ability to induce subsequent changes in synaptic plasticity (Coan et al. 1989). This may be due to a change in the dynamics of activation of calcium calmodulin kinase (CaM KII) and calcium/calcineurin (phosphatase 2B) by high and low levels of intracellular calcium, respectively (Mulkey et al. 1993, 1994). Synaptic desensitization of the NMDA receptor and down regulation of receptor function is known to depend on activation of calcium/calcineurin (Tong et al. 1995). Aβ has also been reported to inhibit the late phase of LTP in vitro via a calcium/calcineurin-dependent mechanism (Chen and Xie 1999). Recently, the p38 MAP kinase pathway has been implicated in the inhibitory actions of Aβ[25–35] on LTP in vitro (Saleshando and O’Connor 2000).

Icv injection of the active fragment of Aβ may then cause disruption of calcium homeostasis (Mattson et al. 1993), including increased activation of the calcium-permeable NMDA channel and/or increased calcium influx directly or via the L-type calcium channel. The subsequent induction of LTP would then be precluded due to changes in the level of channel phosphorylation. Aβ has also been shown to increase calcium

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**FIG. 3.** A: effects of the neurotoxic fragment Aβ[25–35] injected at 10 and 100 nmol, 1 h before the application of tetanic stimulation. Aβ[25–35] at either 10 or 100 nmol did not affect low-frequency baseline synaptic transmission. LTP was significantly reduced by 10 nmol Aβ[25–35] (a; 129 ± 9%, n = 6, P < 0.001) compared with control (vehicle) injected rats (c; 188 ± 15%, n = 7) and blocked by 100 nmol [25–35] (c; 108 ± 15%, n = 6, P < 0.001). EPSPs (averaged, n = 3) recorded 5–10 min pretetanus and 30 min posttetanus are shown for control (vehicle; 1, 2); 10 nmol [25–35] (3, 4) and 100 nmol [25–35] (5, 6). Calibration bars: 1 mV; 5 ms.

**FIG. 4.** A: effect of reversing the sequence of the active neurotoxic peptide on baseline synaptic transmission and LTP. Aβ[35–25] at 10 and 100 nmol had no effect on baseline EPSPs recorded for up to 1 h post icv injection. LTP was significantly reduced following 10 nmol, Aβ[35–25] (a; 136 ± 3%, n = 6, P < 0.01) and 100 nmol Aβ[35–25] (c; 144 ± 7%, n = 8, P < 0.05) compared with control (vehicle) (c; 191 ± 17%, n = 6). Averaged EPSPs (n = 3) are shown recorded 5 min pretetanus and 30 min posttetanus. Control (vehicle; 1, 2); Aβ[35–25], 100 nmol (3, 4) and 10 nmol (5, 6). Calibration bars: 1 mV; 5 ms.
influx via the N-type calcium channel in cerebellar granule cells (Price et al. 1998).

It is unlikely that the deficit in LTP observed following our acute application of low concentrations of β-amyloid peptides is due to cytotoxic processes (Games et al. 1992). The full sequence Aβ[25–35] has been shown to be necessary for peptide aggregation, with an emphasis on the importance of methionine residue (M35) for the promotion and/or stabilization of Aβ aggregation. This property also correlates with the neurotoxic effects of this peptide on cultured cells (Pike et al. 1995). Although we have shown that Aβ[35–25] causes a deficit in LTP in vivo, it has been shown to have no effect on LTP in vitro (Chen et al. 2000). The reversal of the amino acid sequence in Aβ[35–25] alters the aggregation properties of the peptide from β-sheet to random coil formation, thereby reducing the neurotoxic effects on cultured cells (Buchet et al. 1996). It has, however, been reported previously that other peptides that are non-aggregating and non-toxic in cell culture can produce a deficit in LTP in vitro. This suggests that it may not be necessary for a peptide to aggregate to disrupt synaptic plasticity (Chen et al. 2000). In our study, the lower 10-nmol dose of Aβ[35–25] had a greater inhibitory effect than the 100-nmol dose; however, when LTP was measured 30 min following induction, there was little difference in the degree of potentiation observed with either dose. The results obtained using Aβ[35–25] merit further investigation with attention to how this peptide might affect either NMDA receptor–mediated responses or activity of the L-type Ca2+ channel. Our results suggest that the lipophilic portion of the peptide (in the forward or reverse sequence) can impair LTP. In contrast, Aβ[15–25] did not alter LTP when compared with controls. This peptide, unlike Aβ[25–35] and Aβ[35–25], does not contain the lipophilic fragment of Aβ[1–40/42] that is found in senile plaques of AD patients. This was a good indication that icv injections of peptide did not have an unspecified blocking action on LTP.

The Aβ-peptides tested had no effect on low-frequency synaptic transmission recorded during a period of up to 1 h post icv injection. In addition, there was no significant change in PPF. Changes in PPF can be associated with alterations in presynaptic release; increases in PPF indicating a decrease in basal neurotransmitter release. In addition, any change in interneuron activity in vivo could also cause an alteration in PPF. Our results suggest that the Aβ-peptides tested had a negligible effect on interneuron activity or mechanisms of neurotransmitter release in the CA1 region in vivo. There was, however, a trend toward a decrease in PPF that may suggest that Aβ-peptides have subtle effects on these processes.

Our results suggest that Aβ has an effect on the processes involved in LTP in a short period of time, since injection of 100 nmol Aβ[25–35] only 5 min before the application of tetanic stimulation to induce LTP causes a change of LTP to short-term potentiation. This model of in vivo synaptic plasticity is therefore extremely sensitive to β-amyloid. It is possible that this system could provide a working model with which to investigate the mechanism of action of Aβ-peptides and to develop possible therapeutic interventions for the treatment of Aβ-induced neurotoxicity, found in Alzheimer’s disease. These results support the theory that β-amyloid may be responsible at least in part, for the neurodegeneration and decline in cognition associated with Alzheimer’s disease.

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