Impairment of Hippocampal CA1 Heterosynaptic Transformation and Spatial Memory by β-Amyloid_{25–35}

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Sun, Miao-Kun and Daniel L. Alkon. Impairment of hippocampal CA1 heterosynaptic transformation and spatial memory by β-amyloid_{25–35}. J Neurophysiol 87: 2441–2449, 2002. 10.1152/jn.00230.2001. In Alzheimer’s disease, the cholinergic damage (reduced neurotransmission) and cognitive impairment occur long before β-amyloid (Aβ) plaque formation. It has not been established whether the link between soluble Aβ and cholinergic functions contributes to synaptic dysfunction that underlies the cognitive impairment. Here, we report that Aβ_{25–35}, an active form of Aβ, inhibited long-term synaptic modification that depends on the associative activation of cholinergic and GABAergic inputs when bilaterally injected intracerebroventriculally (icv; 200 μg/site). The Aβ microinjections did not affect single-pulse–evoked glutamatergic and GABAergic synaptic transmission onto the hippocampal CA1 pyramidal cells, while cholinergic intracellular θ was dramatically reduced by the Aβ_{25–35} injection. Spatial memory of the water maze task was also impaired by the bilateral icv Aβ_{25–35} injections, while bilateral microinjections of the same dose of Aβ_{1–40} was ineffective in affecting the long-term synaptic modification evoked by associative activation of cholinergic and GABAergic inputs, the cholinergic intracellular θ, or producing memory impairments. Thus restoring the synaptic plasticity involved in this associative activation of cholinergic and GABAergic inputs may offer an important therapeutic target in the treatment of early Aβ-induced memory decline.

INTRODUCTION

Although much of Alzheimer’s disease research has focused on β-amyloid (Aβ) plaque formation in the brain and the neurotoxic effects of Aβ are often associated with its fibrillar or aggregated form, evidence is accumulating that soluble Aβ might produce its cognitive effects independent of plaque formation. First, in patients with Alzheimer’s disease, cognitive deficits occur early, long before plaque formation (Naslund et al. 2000), and some individuals have neuritic plaques but do not show the cognitive deficits associated with Alzheimer’s disease (Hardy 1997). Second, early cognitive effects have also been seen in transgenic animals, in which behavioral deficits precede amyloid deposition (Giacchino et al. 2000; Hsia et al. 1999; Moechars et al. 1999; Mucke et al. 2000). Thus memory deficits are not always associated with significant neuronal cell death in the brain. Third, although initial studies suggested that only fibrillar amyloid was neurotoxic (Lorenzo and Yankner 1994), recent reports (Etcheber-Rigay et al. 1994; Roher et al. 1996) suggest that the soluble oligomeric form may also be neurotoxic. Finally, soluble Aβ_{1–42}, the amyloid-β comprising residues 1–42, was shown to have an immediate dysfunctional effect on nicotinic acetylcholine receptors in hippocampal CA1 interneurons (Pettit et al. 2001).

Memory is widely believed to be expressed by long-lasting modifications of synaptic strengths in relevant neural circuits in the brain. Loss of this synaptic modifiability, although less well studied, may underlie memory disruption in early Alzheimer’s disease. In mammals, the hippocampus is a critical neural structure in early stages of memory formation. In rat hippocampus, temporal interaction of cholinergic and GABAergic inputs, as well as the glutamatergic inputs, received by the CA1 pyramidal cells has been found to result in a rapid postsynaptic transformation (from hyperpolarizing to depolarizing responses) of the GABA_A responses (Collin et al. 1995; Sun et al. 2001a). This type of synaptic transformation that depends on co-stimulation of multiple synapses with different types of transmitters, i.e., heterosynaptic transformation, when induced, alters signal transfer and signal-noise ratio through the CA1 neural network. The synaptic switch depends on an increased bicarbonate formation (sensitive to carbonic anhydrase activity) and conductance through the GABA_A receptor-channel complex (Staley et al. 1995). This bicarbonate-dependent temporal long-term synaptic transformation (LTT) appears to be important in the hippocampus-dependent memory. Inhibition of the bicarbonate formation dramatically reduces the hippocampal θ activity (Sone et al. 1998), a synchronized activity that is believed to gate or facilitate memory information processing in the hippocampus (Teschke and Karhu 2000; Thompson and Best 1989; Winson 1978). Inhibition of the heterosynaptic LTT through inhibition of carbonic anhydrase impairs rats’ ability to learn and memorize the spatial water maze (Sun et al. 2001b). It has not been previously shown, however, that this heterosynaptic LTT is a sensitive indication of Aβ-induced damage. We report here that impairment of the heterosynaptic LTT was observed without obvious abnormality of basal glutamatergic and GABAergic synaptic transmission onto the hippocampal CA1 pyramidal cells. The impairment was associated with reduced ability of the hippocampal CA1 circuits to generate θ activity and to learn a spatial water maze. Thus heterosynaptic LTT may be an important target in soluble...
β-amyloid impairment of the hippocampus-dependent spatial memory, and possibly early Alzheimer’s disease.

METHODS

Spatial maze tasks

Effects of Aβ25-35, the amyloid-β comprising residues 25–35, in vivo on spatial memory were evaluated in rats with the Morris water maze task (Meiri et al. 1998; Sun et al. 2001b). Male adult Wistar rats (180–200 g) were housed in a temperature-controlled (20–24°C) room for 1 wk, allowed free access to food and water, and kept on a 12-h light/dark cycle. Rats were anesthetized with pentobarbital sodium (60 mg/kg ip) and placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). The core temperature of rats was monitored and kept constant (38.0 ± 0.5°C) with warming light and pad. Two stainless steel guide cannulas were placed with the tips positioned at the coordinates (anterior-posterior, 0.5 mm; lateral, 1.5 mm; horizontal, 3.2 mm), under aseptic conditions. At the end of surgery and under appropriate anesthesia, rats received bana- mine (1 mg/kg sc) and ketoprofen (5 mg/kg sc). A 7-day recovery period was allowed before any further experimentation.

On the first day of experiments, rats were randomly assigned to different groups (20 each) and bilaterally and intracerebroventricularly (icv) injected with either Aβ25-35, or Aβ1–12 (Bachem Bioscience, King of Prussia, PA; 200 μg/2 μl/2 min/site; prepared just before use), or the same volume of vehicle. Aβ1–12, a 11 amino acid with a sequence that was the reverse of Aβ25-35, was used to test the specificity of amino acid sequence of Aβ25-35. Ten rats of each group were used for the water maze performance, while the rest for in vitro examination of synaptic function of the hippocampal CA1 neurons with the same time frame.

On the third day after the injections, rats swam for 2 min in a 1.5-m (diam) × 0.6-m (depth) pool; 22 ± 1°C. On the following day, rats were trained in a 3 trial per day task for 3 consecutive days. Each training trial lasted for up to 2 min, during which rats learned to escape from water by finding a hidden platform that was placed at a fixed location and submerged about 1 cm below the water surface. The navigation of the rats was tracked by a video-camera. The escape latency and the route of rats’ swimming across the pool to the platform were recorded. The quadrant test (1 min) was performed after removing the platform, 24 h after the last training trial.

Hippocampal slice electrophysiology

Rats were decapitated, and the brains were removed and cooled rapidly in an artificial cerebrospinal fluid (ACSF) solution (≈4°C), bubbled continuously with 95% O2–5% CO2. Hippocampi were sliced (400 μM) into 124 NaCl, 3 KCl, 1.3 MgSO4, 2.4 CaCl2, 26 NaHCO3, 1.25 NaH2PO4, and 10 glucose; and perfused (2 ml/min) with the oxygenated ACSF in an interface chamber at 30–31°C. Intracellular recordings were obtained from CA1 pyramidal neurons using glass micropipettes filled with 2 M potassium acetate (pH 7.25), with measured tip resistance in the range 70–120 MΩ. Cells that showed obvious accommodation, an identifying characteristic of pyramidal cells, were used in the study. Labeling the recorded cells exhibiting this characteristic with dye has previously revealed that the recorded cells are indeed pyramidal cells (Sun et al. 1999). Signals were amplified, digitized, and stored using AxoClamp-2B amplifier and DigiData 1200 with the P-clamp data acquisition and analysis software (Axon Instruments, Foster City, CA). Stable GABAergic inhibitory postsynaptic response (IPSP) or glutamatergic excitatory postsynaptic response (EPSP) could be evoked for several hours without noticeable change in amplitudes. Stratum pyramidale and Stratum oriens were stimulated (about 280 μm lateral and dorsal from the recording electrode, respectively), using bipolar electrodes constructed of Teflon-insulated PtIr wire (25 μm diam, the approximate thickness of Stratum pyramidale; FHC, Bowdoinham, ME). The Stratum oriens stimulating electrode was about 400 μm from the other stimulating electrode and was used to activate cholinergic terminals. Stimulation delivered into Stratum oriens evoked acetylcholine release and postsynaptic responses that were enhanced by eserine and blocked by atropine (Cole and Nicoll 1984), in the pyramidal cells. The effects of microstimulation of Stratum oriens on heterosynaptic LTD were eliminated by 20 μM atropine, which did not affect IPSPs elicited by single-pulse stimulation of Stratum pyramidale (Sun et al. 2001a), indicating an involvement of muscarinic receptor activation. Co-stimulation of Stratum oriens and Stratum pyramidale consisted of single pulses stimulation of Stratum oriens (20–60 μA and 50 μs, 1 Hz for 30 s) and stimulation of Stratum pyramidale (10 trains, 10 pulses at control intensity (30–60 μA and 50 μs) and at 100 Hz, a 0.5-s inter-train interval) at the last 6 s of Stratum oriens stimulation (Sun et al. 2001a). In some experiments, a stimulating electrode was placed in the stratum radiatum to stimulate the Schaeffer collateral glutamatergeic pathway. Test stimuli were applied at 1 per minute (0.017 Hz). Experiments in which >20% variation in the evoked IPSP or EPSP magnitudes occurred during 10 min control period were discarded.

Statistical analysis was performed using the Student’s t-test for paired or unpaired data or ANOVA whenever appropriate. The values are expressed as means ± SE of the mean, with n indicating number of the cells or rats. All animals used in these experiments were treated under National Institutes of Health guidelines for the welfare of laboratory animals.

RESULTS

We tested the effects of Aβ25-35 on spatial learning in rats, using the hidden-platform water maze. As shown in Fig. 1A, the latency to escape to the platform in all three groups of rats decreased following the training sessions, indicating that all rats showed some degree of learning. But the speed and the extent of the learning were significantly different. Statistical analysis revealed significant effects of groups (F2,27 = 6.72, P < 0.01), trials (F8,247 = 3.72, P < 0.001), and groups × session of trials (F6,247 = 2.49, P < 0.001), indicating that spatial learning in rats injected with Aβ25-35 (Aβ25-35 rats) was slower than that in rats injected with Aβ1–12 (Aβ1–12 rats), or vehicle (control rats). Moreover, a post hoc analysis reveals a significant difference from the second trials (P < 0.05), confirming worse learning in Aβ25-35 rats. In fact, the escape latency of the Aβ25-35 rats did not reach the same level of plateau (Fig. 1A). In our study, Aβ35–25, an 11 amino acid with a sequence that was the reversal of ordering of Aβ25-35 was used as a control to test the specificity of the toxic amino acid sequence of Aβ25-35. There was no difference (F1,18 = 1.52, P > 0.05) between the Aβ35–25 and vehicle groups (Fig. 1A). The same extent of plateau for escape latency was also reached in the Aβ35–25 and vehicle groups (Fig. 1A), indicating that Aβ25-35 injection did not produce any detectable effects on the spatial memory. Thus Aβ25-35, when injected into the lateral ventricle, induced an early significant reduction in rats’ ability to solve the spatial water maze. The average swim speeds for all nine trials, however, did not differ between all the groups (Fig. 1B; P > 0.05), indicating that injections of the peptides and vehicle did not grossly affect their sensory or locomotor activities. During the experimental periods, no rats showed any apparent sign of discomfort or abnormal behaviors such as hypo- or hyperactivity.

Quadrant tests 24 h after the last training trial revealed that
FIG. 1. Aβ25-35 impairs rat spatial memory in vivo. A: the mean ± SE escape latency across 9 trials in the water maze by rats given a single dose (intracerebroventricularly; see METHODS) of Aβ25-35, Aβ35-25, or vehicle. B: swim speeds over the 9 trials reveal no significant difference between the groups. C–E: quadrant preference of Aβ25-35 (C), Aβ35-25 (D), or vehicle (E)-injected rats (in 1 min). A platform for escape was placed in quadrant 4 during the training trials. Insets are paths taken by representative rats with quadrant numbers indicated.
the $\text{AB}_{2.35-35}$ rats ($F_{3.36} = 1.57$, $P > 0.05$; ANOVA and Newman-Keuls post hoc test) did not show significant preference (Fig. 1C) for the target quadrant (Quadrant 4) where the platform was previously placed during training trails and had been removed, while the $\text{AB}_{2.35-35}$ rats ($F_{3.36} = 197.2$, $P < 0.0001$; Fig. 1D) and vehicle rats ($F_{3.36} = 169.5$, $P < 0.0001$; Fig. 1E) spent more time searching in the target quadrant. However, in comparison with vehicle, $\text{AB}_{2.35-35}$ rats exhibited a similar preference for the target quadrant ($P > 0.05$; unpaired t-test).

Hippocampal slices were prepared on the 4th to 6th days after the administration of $\text{AB}_{2.35-35}$- or vehicle. Effects on the synaptic plasticity, heterosynaptic GABAergic LTT, intracellular $\theta$, and long-term synaptic potentiation (LTP) of the glutamatergic inputs into the hippocampal CA1 pyramidal cells, were examined. In general, there were no obvious differences in terms of cell numbers per recording session (thus probable cell density), membrane potentials, or impedance between the groups when the recordings were stabilized.

Microstimulation of Stratum pyramidale evoked an IPSP recorded in the pyramidal cells. There was no differences ($P > 0.05$) as to the magnitudes of the IPSPs evoked with the same intensity ($50 \mu A, 50 \mu s$) of stimulation and recorded in the hippocampal CA1 pyramidal cells from the $\text{AB}_{2.35-35}$ (peak IPSPs: $-7.9 \pm 0.6$ mV, $n = 24$), $\text{AB}_{3.25-35}$ (peak IPSPs: $-7.7 \pm 0.9$, $n = 18$), or vehicle rats (peak IPSPs: $-7.9 \pm 0.8$, $n = 15$), indicating that a few days after the $\text{AB}_{2.35-35}$ injection, presynaptic (GABAergic interneurons), postsynaptic (pyramidal cells) structures/mechanisms, and synaptic transmission remain functionally normal. As described previously (Sun et al. 1999, 2001b), the IPSPs were not altered by application of 500 $\mu M$ kynurenic acid (30 min), a wide spectrum glutamate receptor antagonist that blocks both the $N$-methyl-$d$-aspartate (NMDA) and non-NMDA receptors (Collingridge and Lester 1989; Sun 1996), but the IPSPs were eliminated by 1 $\mu M$ bicuculline (not shown), a GABA$_A$ receptor antagonist. This concentration of kynurenic acid was sufficient to eliminate the glutamatergic IPSPs, evoked by stimulation of the Schaffer collateral pathways in the CA1 pyramidal cells (Sun et al. 1999). Thus the IPSPs were mediated mainly, if not exclusively, by the GABA$_A$ responses.

Co-stimulation of the cholinergic and GABAergic inputs induced LTT of the GABAergic postsynaptic responses (Collin et al. 1995; Sun et al. 2001a), a response sensitive to a blockade with the muscarinic receptor antagonist atropine (Sun et al. 2001a). Heterosynaptic LTT of the GABAergic responses was induced by co-stimulation of Stratum oriens and Stratum pyramidale in the slices from $\text{AB}_{3.25-35}$ and vehicle rats. These effects lasted more than 1 h (Fig. 2A). For instance, 50 min after the co-stimulation, the peak GABAergic responses were significantly ($P < 0.05$) and similarly reversed (post-$\text{AB}_{3.25-35}$: $5.2 \pm 0.8$ mV, vs. pre-$\text{AB}_{3.25-35}$: $-7.3 \pm 0.9$ mV, $n = 9$; postvehicle: $5.4 \pm 0.6$ mV, vs. prevehicle: $-7.6 \pm 0.6$ mV, $n = 8$), when recorded at their resting membrane potentials, in the two groups as compared with their pre–co-stimulation values (Fig. 2B). Peak responses, instead of assigned isochronal points during the time course of the IPSPs, were compared because the response time course and time to maximum response were found to depend on the direction of anion flux in a previous study (Sun et al. 2001a). The reversed postsynaptic GABAergic response exhibited a shorter latency to peak and duration than the IPSPs, a phenomenon that may depend on the direction of ionic flow and/or channel kinetics (Sun et al. 2001a). The heterosynaptic LTT, however, was impaired in the $\text{AB}_{2.35-35}$ rats (Fig. 2A). The same intensity of co-stimulation of Stratum oriens and Stratum pyramidale did not induce the synaptic switch of the GABAergic postsynaptic response in the $\text{AB}_{2.35-35}$ rats. The IPSPs were only slightly reduced in magnitudes (post-$\text{AB}_{3.25-35}$: $-5.6 \pm 0.7$ mV, vs. pre-$\text{AB}_{3.25-35}$: $-7.5 \pm 0.8$ mV, $n = 9$; $P < 0.05$) for instance, 50 min after the co-stimulation as compared with those pre–co-stimulation values (Fig. 2B).

Bath application of carbachol ($50 \mu M$, 20 min), a cholinergic receptor agonist, to hippocampal slices mimicked diffuse transmission by acetylcholine from septal activation (Descaries et al. 1997) and induced the intracellular $\theta$ in the hippocampal CA1 pyramidal cells from $\text{AB}_{3.25-35}$ rats (Fig. 3B; peak amplitude: $6.1 \pm 0.5$ mV, 50 min after the cholinergic activation, $n = 4$, $P < 0.05$, from background noise) and vehicle rats (not shown). The $\theta$ activity was sensitive to atropine blockade and lasted for more than 3 h, as reported by others (Huerta and Lisman 1995). In the pyramidal cells from the $\text{AB}_{2.35-35}$ rats, however, the same cholinergic activation ($50 \mu M$ carbachol, 20 min) did not induce a significant intracellular $\theta$ (Fig. 3A) during a 3-h postcarbachol recording period. The $\theta$-like oscillation of membrane potential was an averaged magnitude of $0.9 \pm 0.5$ mV (50 min after the cholinergic activation; $n = 3$; $P > 0.05$).

Stimulation of the Schaffer collateral pathway evoked a glutamatergic EPSP in the hippocampal CA1 pyramidal cells. The EPSPs observed in the hippocampal CA1 pyramidial cells from the $\text{AB}_{3.25-35}$ rats (peak EPSPs: $5.5 \pm 0.4$ mV, $n = 22$) did not differ ($P > 0.05$) from those of $\text{AB}_{3.25-35}$ (peak EPSPs: $5.3 \pm 0.5$, $n = 20$), or vehicle rats (peak EPSPs: $5.4 \pm 0.5$, $n = 18$), evoked with the same intensity ($30 \mu A, 50 \mu s$) of stimulation, indicating that the basal glutamatergic synaptic transmission was functionally normal 4–6 days after the $\text{AB}_{3.25-35}$ injection.

The LTP of the Schaffer collateral glutamatergic inputs into the hippocampal CA1 cells did not appear to be impaired in any of the three groups. High-frequency stimulation (100 Hz, 1 s) of the Schaffer collateral pathway induced LTP that lasted longer than 1 h (Fig. 4A). For instance, 50 min after the LTP induction, the EPSPs were all significantly bigger ($P < 0.05$) than their prestimulation values (post-$\text{AB}_{3.25-35}$: $9.2 \pm 0.7$ mV, vs. pre-$\text{AB}_{3.25-35}$: $5.6 \pm 1.1$ mV, $n = 8$; post-$\text{AB}_{3.25-35}$: $8.9 \pm 1.1$ mV, vs. pre-$\text{AB}_{3.25-35}$: $5.2 \pm 0.8$ mV, $n = 7$; postvehicle: $9.1 \pm 0.9$ mV, vs. prevehicle: $5.4 \pm 0.6$ mV, $n = 6$), when recorded at their resting membrane potentials (Fig. 4B).

**DISCUSSION**

The major findings of the present study are that a single dose of $\text{AB}_{2.35-35}$ produced an early impairment of water maze spatial memory that was also associated with impaired heterosynaptic LTT of the GABAergic responses of the hippocampal CA1 pyramidal cells and impaired generation of cholinergic $\theta$ of the CA1 pyramidal cells. These impairments occurred at the time when basal synaptic transmission of the GABAergic and glutamatergic inputs onto the CA1 pyramidial cells and LTP of Schaffer collateral glutamatergic inputs were not significantly impaired. Thus the effects were unlikely to have resulted from widespread apoptosis/neural injury. The results suggest that the
heterosynaptic interaction and intracellular activity of the CA1 pyramidal cells are more sensitive indicators of the β-amyloid-induced memory impairment than the LTP and may indeed play an important role in the hippocampal-dependent spatial memory.

Encoding experiences into memory may involve a diversity of synaptic plasticity (Ahrens and Freeman 2001; Brenowitz et al. 1998; Kornhauser and Greenberg 1997; Otis et al. 1996; Paulsen and Moser 1998), including changing the operation of preexisting synapses and the growth of new synapses. The GABAergic postsynaptic depolarization can be induced by several different paradigms (Alkon et al. 1992, 1998; Kaila et al. 1993, 1997; Michelson and Wong 1991; Siklós et al. 1995; Staley et al. 1995; Sun et al. 1999). The flux of anions through the GABA receptor channel complex depends on anionic gradients for chloride and bicarbonate (Kamermans and Werblin 1992; Kulik et al. 2000) and is sensitive to many modulators and neurotransmitters (Alvarez and Banzan 1990; Morrow et al. 1988). Histamine, a well-established carbonic anhydrase activator, is long known to have behavioral effects (Alvarez and Banzan 1990; Li et al. 1990). However, it is not known whether such effects principally involve an action of histamine.
on carbonic anhydrase. In the rat olfactory glomeruli, local Cl\textsuperscript{−} redistribution may be responsible for reversed GABA responses at the GABA\textsubscript{A} receptor channel complex (Sikl\’os et al. 1995). The “Cl\textsuperscript{−} shift” may also affect synaptic levels of neurotransmitters, such as dopamine (Amiedjki-Chab et al. 1992). In red blood cells, chloride influx (“shift” of ions) can offset significant efflux of bicarbonate secondary to intracellular CO\textsubscript{2} elevation. In the brain, CO\textsubscript{2} elevation accompanies glucose utilization that increases in those neurons with increased impulse activity and therefore increased requirements for energy, ultimately in the form of ATP. We have previously suggested that GABA\textsubscript{A}ergic inhibition transformed into excitation may enhance attention, i.e., increase the contrast between the “relevant” and “irrelevant” information in “attentional space.” The septal cholinergic pathway in the hippocampus mediates attention for learning new information. Therefore the cholinergic-GABA\textsubscript{A}ergic LTT, found here to be particularly vulnerable to β-amyloid toxicity, may increase impulse activity in those neurons receiving relevant afferent sensory inputs. Increased activity, glucose utilization, CO\textsubscript{2} elevation, and bicarbonate efflux might then be expected to cause further LTT in a possible feedback cycle. Such a feedback cycle could be critical for attentional focus that shifts to different ensembles of neurons (in a structure such as the hippocampus) to adapt in a dynamic fashion to shifting behavioral requirements of the organism. A dysfunctional attentional process could then disrupt recent memory as a result of soluble Aβ toxicity, as typically occurs in early Alzheimer’s disease.

In the hippocampus, the mechanism underlying reversed GABA postsynaptic response involves an enhanced HCO\textsubscript{3}\textsuperscript{−} conductance through GABA\textsubscript{A} receptor channels and may participate in the generation of synchronized neural activity such as hippocampal θ rhythm (Sun et al. 2001a), which is believed by many to gate or facilitate memory information processing in the hippocampus. The importance of GABA\textsubscript{A}ergic interneurons in the information processing through the hippocampal network is indicated by the fact that one basket interneuron selectively and perisomatically innervates approximately 1,000 pyramidal cells (Buhl et al. 1994; Cobb et al. 1995) and can entrain a large population of the principal cells. They are particularly active and express strongest rhythmic θ discharges when hippocampal electroencephalograph (EEG) is dominated by θ rhythm (Paulsen and Moser 1998). The net change from a hyperpolarizing response to depolarizing response is a rapid change from an excitatory filter to an excitatory amplifier, thus altering signal transfer through the network (Sun et al. 1999).

Damaging the ability of the network by Aβ\textsubscript{25–35} to induce the heterosynaptic GABA\textsubscript{A}ergic depolarization prevents the networks’ efficient and dynamic control of signal transfer. The basis for this acute impairment is unclear but appears to involve cholinergic signal cascades. It is well established that a selective dysfunction of cholinergic neurons that originate in the basal forebrain and project to the cortex and hippocampus is a characteristic feature of Alzheimer’s disease (Bartus et al. 1982; Winson 1978). The mechanisms underlying the impairment of heterosynaptic interaction were not determined in our study, but may include a reduced cholinergic transmission (the transmitter and receptors) in the network and/or muscarinic cholinergic signal transduction (Kelly et al. 1996), such as impairment of carbonic anhydrase activation and/or of increased bicarbonate flux through the GABA\textsubscript{A} receptor-channel complex. Postsynaptic mechanisms may be partly involved since effects of direct cholinergic activation were also diminished by the Aβ administration. It has been known for some time that Alzheimer’s disease is associated with the reduction/loss of basol cholinergic neurons, presynaptic terminals, high-affinity choline uptake, choline acetyl transferase activity, and a decrease in the number of neuronal acetylcholine receptors (Kar et al. 1998; Wang et al. 2000a,b). Aβ also acutely inhibits potassium-evoked acetylcholine release from rat hippocampal formation (Kar et al. 1996). Direct receptor inhibition is unlikely since Aβ\textsubscript{1–42} did not directly bind to muscarinic receptors at micromolar concentrations (Wang et al. 2000b). Another possibility is that Aβ\textsubscript{25–35} might interfere with functions of carbonic anhydrase, or whose activity the heterosynaptic LTT and θ generation/maintenance depend on. Aβ can be internalized (Bahr et al. 1998). Binding to zinc (Yang et al. 2000) of carbonic anhydrase by the Aβ peptides would produce a potent inhibition of the enzyme activity. The possible involvement of carbonic anhydrase in Aβ and Alzheimer’s neurodegenerative diseases is an interesting hypothesis since it has been known for quite some time that carbonic anhydrase...
activity is significantly lower in the elderly and even lower in Alzheimer’s patients (Meier-Ruge et al. 1984). On the other hand, effects of Aβ25-35 on the basal synaptic transmission and LTP were not evaluated at larger doses or after an extended period. Thus our results do not suggest that these mechanisms are not affected by the Aβ at higher doses or at different times (Chen et al. 2000; Itoh et al. 1999; Wu et al. 1995).

The amyloid precursor protein (APP) is constitutively expressed in many cell types and in the nervous system may function as an important repair protein. The release of Aβs involves cleavage of APP by secretases. The released forms including Aβ1-40 and Aβ1-42, however, are soluble. These soluble Aβs aggregate into the insoluble amyloid fibrils to form amyloid plaques. The process of aggregation, which varies in length depending on Aβ concentrations and environmental factors, is not well understood but appears to involve conformational changes and interaction with other molecular components such as zinc. A common practice to study the effects of aggregated Aβ is “aging,” i.e., preincubation of the soluble Aβ at 37°C for several days before administration (e.g., Hoshi et al. 1997). In our study, the soluble form was administered.

We have shown that Aβ25-35 administration caused an early impairment of rat water maze spatial memory and a damaged

![Graph showing time course of EPSPs for different conditions](http://jn.physiology.org/)

**FIG. 4.** Aβ25-35 does not impair LTP of glutamatergic inputs in hippocampal CA1 pyramidal cells. A: time course of the Schaffer collateral pathway–CA1 glutamatergic postsynaptic responses recorded in the hippocampal CA1 pyramidal cells from Aβ25-35, Aβ35-25, or vehicle-injected rats. The 1st vertical arrow indicates the time when high-frequency stimulation of the Schaffer collateral pathway was applied. B: responses of glutamatergic postsynaptic responses before (left 3 columns) and 50 min after (right 3 columns) LTP induction (see METHODS). Examples of the recorded postsynaptic responses are illustrated (right inset) as pre- (1) and post- (50 min) stimulation (2) in slices obtained from Aβ25-35 (top), Aβ35-25 (middle), or vehicle (bottom)-injected rats. Arrowheads indicate the time when single pulse Schaffer collateral stimulation was delivered. *P < 0.05.
heterosynaptic LTT. Thus the ability to generate hippocampal $\theta$ and postsynaptic GABA depolarization was impaired at the time when the spatial memory was damaged. Basal synaptic transmission within the same brain area and LTP induction of the glutamatergic pathway, however, appeared to remain intact. The most reasonable explanation for our results is an essential requirement of heterosynaptic interaction in the signaling pathways for learning and memory. Reduced cholinergic contributions to a Ca$_{\text{v}}^{2+}$-dependent increase in bicarbonate formation and/or conductance through the GABA$_{A}$ receptor-channel complex might be involved, although our study does not establish that the GABAergic depolarization, as defined in vitro, directly contributes to spatial memory. This explanation, however, is consistent with the occurrence of mental retardation in carbonic anhydrase II–deficient patients (Sly and Hu 1995) and effects of carbonic anhydrase inhibitor on the hippocampal $\theta$ (Sone et al. 1998) and the spatial learning and memory (Sun et al. 2001b). Thus this heterosynaptic interaction may represent an important therapeutic target in restoring $\beta$-induced memory decline and possibly for ameliorating mental retardation.

REFERENCES


