β-Amyloid-(1–42) Impairs Activity-dependent cAMP-response Element-binding Protein Signaling in Neurons at Concentrations in Which Cell Survival Is Not Compromised*

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Cognitive impairment is a major feature of Alzheimer’s disease and is accompanied by β-amyloid (Aβ) deposition. Transgenic animal models that overexpress Aβ exhibit learning and memory impairments, but neuronal degeneration is not a consistent characteristic. We report that levels of Aβ-(1–42), which do not compromise the survival of cortical neurons, may indeed interfere with functions critical for neuronal plasticity. Pretreatment with Aβ-(1–42), at sublethal concentrations, resulted in a suppression of cAMP-response element-binding protein (CREB) phosphorylation, induced by exposure to either 30 mM KCl or 10 μM N-methyl-D-aspartate. The effects of Aβ-(1–42) seem to involve mechanisms unrelated to degenerative changes, since Aβ-(25–35), a toxic fragment of Aβ, at sublethal concentrations did not interfere with activity-dependent CREB phosphorylation. Furthermore, caspase inhibitors failed to counteract the Aβ-(1–42)-evoked suppression of CREB activation. Aβ-(1–42) also interfered with events downstream of activated CREB. The Aβ-(1–42) treatment suppressed the activation of the cAMP response element-containing brain-derived neurotrophic factor (BDNF) exon III promoter and the expression of BDNF exon III mRNA induced by neuronal depolarization. In view of the critical role of CREB and BDNF in neuronal plasticity, including learning and memory, the observations indicate a novel pathway through which Aβ may interfere with neuronal functions and contribute to cognitive deficit in Alzheimer’s disease before the stage of massive neuronal degeneration.

Alzheimer’s disease (AD)1 is identified by a progressive decline in cognitive functions and is characterized by neuropathologies including senile plaques comprising β-amyloid (Aβ) peptide, neurofibrillary tangles, and ultimately neuron loss. Mutations in the Aβ precursor protein (APP) and in the prese-nilin (PS1 and -2) genes have been identified, which co-segregate with familial Alzheimer’s disease (FAD) (1, 2). Overexpression of the mutated genes in cells and in transgenic animals has been shown to mimic some of the features of AD, including increased accumulation of Aβ (1, 3–5). Most significantly, transgenic mice also show impairments in neuronal function (5) and develop deficits in learning and memory (4), although neuron loss is not a consistent feature of the phenotype (4–7). Although it is known that Aβ is neurotoxic in vivo and in vitro (8), there are indications that cognitive impairment may precede both high levels of Aβ accumulation and pronounced neuronal degeneration in the brain (5, 9–11). Because of the proactive and progressive nature of the disease, Aβ may be present in the brain at sublethal concentrations for extended periods. Although at these levels Aβ does not compromise neuron survival, it may affect critical signal transduction processes that mediate plastic neuronal changes, including those involved in learning and memory. The transcription factor CREB, which regulates expression of cAMP response element (CRE)-containing genes, plays an essential role in learning and memory processes in a variety of species ranging from Drosophila to mammals (12–15). Phosphorylation at Ser-133 is critical for the transcriptional activity of CREB (16–18). Neuronal activity-dependent phosphorylation of Ser-133 of CREB has been well documented, and in cultured neurons, both NMDA receptor activation and membrane depolarization can lead to the activation of CREB (17–19). Disruption of CREB function specifically interferes with activity-dependent synaptic plasticity ranging from long term potentiation (LTP) to long term memory (13–15). It is expected, therefore, that mechanisms that interfere with CREB activation would compromise CREB activity-dependent neuronal function through disruption of downstream gene expression.

Brain-derived neurotrophic factor (BDNF) is one of the target genes of CREB (17, 18). BDNF, a member of the neurotrophin family, enhances survival, differentiation, and growth of certain neuronal populations, modulates synaptic activity, and acts as an effector of neuronal plasticity both during development and in the adult (20, 21). BDNF participates in LTP, is up-regulated in the hippocampus during learning (22), and deficits in BDNF compromise LTP and learning and memory (20). BDNF mRNA and protein are reduced in the hippocampus in AD (23–25), a reduction proposed to contribute to cognitive decline observed in AD. Thus, examination of BDNF transcription provides a means of assessment of effects on CREB regulation, which may play a significant role in the pathogenesis of AD.

Here, we report that levels of Aβ-(1–42), which do not affect the survival of cortical neurons, may indeed interfere with functions critical for neuronal plasticity, by eliciting a reduction of the activity-dependent phosphorylation of CREB and

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‡The abbreviations used are: AD, Alzheimer’s disease; Aβ, β-amyloid; ADDL, Aβ-derived diffusible ligands; APP, amyloid precursor protein; BDNF, brain-derived neurotrophic factor; CREB, cAMP-response element-binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NMDA, N-methyl-D-aspartate; FAD, familial Alzheimer disease; PBS, phosphate-buffered saline; RT-PCR, reverse transcriptase-polymerase chain reaction; z-VAD-fmk, benzoyloxycarbonyl-Ala-Aspfluoromethyl ketone; CRE, cAMP response element; LTP, long term potentiation; DIV, days in vitro.

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the expression of BDNF, one of the important target genes of this transcription factor.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**— Cultures greatly enriched in cortical neurons from embryonic day 18 rat fetuses were prepared as described previously (26). Cells plated at 2.5 × 10⁴ cells/cm² were cultured in poly-L-lysine-treated multwell plates and maintained in serum-free optimal Dulbecco’s modified Eagle’s medium supplemented with B-27 components (Life Technologies). When cells were exposed to Aβ, the medium was switched to Dulbecco’s modified Eagle’s medium/B27 containing Aβ. Cultures were maintained for 5 days before treatments. Neuronal survival was assessed by trypan blue exclusion (26) and in select experiments using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (27) and annexin V binding to externalized phosphatidylserine to monitor early changes in putative apoptotic processes (28); these methods gave comparable results. Tetrodotoxin (1 μM) was added 2 h before treatments to reduce endogenous synaptic activity. Cells were stimulated using either 30 mM KCl (high K⁺) for 15 min or 10 μM NMDA for 10 min. Amino-5-phosphonovaleric acid (100 μM) was added to the cultures 30 min before the high K⁺ exposure.

**Aβ Preparation**— Aβ peptides were synthesized by solid-phase N-Fmoc (9-fluorenylmethyl) (Fmoc) amino acid chemistry, purified by reverse-phase high performance liquid chromatography, and characterized by electrospray mass spectrometry as described previously (26, 29).

A stock solution of Aβ(1–42) (1 mM) was prepared in distilled water and used after one freeze-thaw cycle. We have documented previously that Aβ(1–42) peptides prepared this way assemble into β-sheet fibrils as determined by thioflavin staining, circular dichroism, electron microscopy, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis and are toxic to nerve cells (26, 29, 30). To facilitate comparisons, preparations of Aβ(1–42) peptides containing all residues from the same lot of Aβ(1–42) peptides as the β-sheet containing Aβ(1–42) preparations described above. Solutions of ADDLs were prepared as described previously (31, 32). In brief, Aβ(1–42)-containing solutions (0.34 mg/ml) were incubated with clusterin (0.17 mg/ml) at 24 °C for 12 h. Solutions were centrifuged at 14 000 × g for 15 min to remove large aggregates, and the supernatant was used for all assays. In agreement with the published features of these preparations, microscopic examination indicated that the supernatant contained no large aggregates.

**Western Blotting**— Cortical neurons were lysed in SDS sample buffer, and the proteins were resolved by SDS–polyacrylamide gel electrophoresis (10% acrylamide) and transferred to polyvinylidene difluoride membrane. Membranes were incubated at room temperature in PBS containing 5% nonfat milk for 60 min to block the nonspecific binding. Following incubation with the primary antibodies specific for either Ser-133-phosphorylated CREB (P-CREB) (1:1,000) or total CREB (T-CREB), which recognize both phospho- and dephospho-CREB, the blots were incubated with the secondary antibody, goat anti-rabbit IgG conjugated with horseradish peroxidase (both antibodies from Upstate Biotechnology, Inc.), the blots were washed in PBS containing 0.1% Tween and then incubated with the secondary antibody, goat anti-rabbit IgG conjugated with horseshadish peroxidase (Vector Laboratories), at 1:8000 dilution in the blocking solution for 60 min. Blots were then washed four times with PBS containing 0.1% Tween. Immunolabeling was detected by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech) according to the recommended conditions. Immunoreactivity was quantified using densitometric analysis.

**Immunocytochemistry**— Cultures were initially treated with a solution of 3% aqueous H₂O₂ for 3 min, then briefly rinsed in Tris-buffered saline (TBS, 0.05 M Tris in 0.9% NaCl, pH 7.4), and incubated in TBS containing 5% normal goat serum. The cultures were then incubated at 4 °C in TBS, 5% serum containing the rabbit polyclonal IgG against P-CREB (1:2000) or total CREB (T-CREB), which recognize both phospho- and dephospho-CREB (1:1000) (both antibodies from Upstate Biotechnology, Inc.), the cultures were rinsed with PBS containing 0.1% Tween and then incubated with the secondary antibody, goat anti-rabbit IgG conjugated with horseshadish peroxidase (Vector Laboratories), at 1:8000 dilution in the blocking solution for 60 min. Blots were then washed four times with PBS containing 0.1% Tween. Immunolabeling was detected by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech) according to the recommended conditions. Immunoreactivity was quantified using densitometric analysis.

**Cortical Neuron Transfection and Luciferase Assay**— Cortical neurons were transfected with plasmid pIII(170)Luc (a kind gift of Dr. Michael E. Greenberg, Harvard Medical School) at 3 DIV using a procedure described previously (33). Briefly, all transfections were conducted in 6-well 35-mm dishes with LipofectAMINE (Life Technologies, Inc.) according to the manufacturer’s instructions. One well was transfected with 1 μg of reporter plasmid and 0.1 μg of pRL-CMV (Promega), a cytomaglovirus-luciferase control plasmid to normalize BDNF exon III promoter activity. Forty hours after transfection, cultures received 30 mM KCI for 9 h and then plates were washed twice with cold PBS, Amersham Pharmacia following the manufacturer’s instructions. Twenty μl of cell extract were used for a dual-luciferase reporter assay (Promega) according to the manufacturer’s instructions.

**RT-PCR—BDNF exon III and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA contents were estimated using RT-PCR. Total RNA was isolated from cultures at 5 DIV by a spin column kit (Promega), and RT-PCR was carried out using a one-tube RT-PCR kit (Amersham Pharmacia) following the manufacturer’s instructions. 100 ng of total RNA was used, and the single-stranded cdNA was amplified by PCR with exon-specific primers. The primers for BDNF exon III and GAPDH were described previously (18). BDNF exon III primers were as follows: reverse primer E5R, 5'-GAGAAGGTGATGACCATCT-3' and forward primer E3F (exon III-specific), 5'-TCCGATATTACCTCCGGCATCT-3'. GAPDH primers were 5'-TCTATGACATTTGCGTATGG-3' and 5'-GGTTGCTTTGAATGTCAGGAG-3'. The amount of total RNA in the samples was normalized to the amount of GAPDH. The PCR products were separated by electrophoresis on 6% polyacryl- mide gels and stained using Vistra Green (Amersham Pharmacia Biotech). The products were quantified by PhosphorImager (Molecular Dynamics) analysis.

**RESULTS**

**Low Concentrations of Aβ(1–42) Decrease Neuronal Activity-Dependent Phosphorylation of CREB—β-Amyloid peptides, such as Aβ(1–42) and Aβ(25–35), are known to compromise neuronal survival both in vitro and in vivo (8, 34). The effect of Aβ(1–42) on cultured neurons is concentration-dependent, and significant cell loss was detected after treatment at concentrations > 20 μM. We also observed that under our experimental conditions there was a threshold for the effect of Aβ(1–42) on cell viability. Twenty-four-hour exposure of cortical neurons to Aβ(1–42) at > 20 μM with the lot of peptide used in this study resulted in significant cell loss, whereas survival was not affected at ≤ 10 μM (not shown). A concentration range of 1–10 μM was therefore chosen for the exploration of the influence of Aβ(1–42) on neuronal function. It should be noted that no studies to date have assessed the effect of sublethal levels of Aβ on the regulation of gene expression that is mediated by activity-dependent signal transduction.

Cognitive dysfunction is a characteristic feature of the AD phenotype. Recent studies have demonstrated that synaptic plasticity, including certain forms of LTP and memory, depends critically on the activation of the transcription factor CREB (15–15). Therefore, we examined the influence of sublethal concentrations of Aβ(1–42) on activity-dependent CREB phosphorylation. K⁺-induced depolarization of neurons is known to elicit the phosphorylation of CREB at Ser-133, an event that is essential for its transcription-activating function (16–18). Therefore, the level of activated CREB was examined with Western blotting using specific antibodies against Ser-133-phosphorylated CREB (P-CREB). Pretreatment with 5 or 10 μM Aβ(1–42) for 1 h decreased high K⁺-induced elevation of the amount of P-CREB (Fig. 1, A and B). Pretreatment with 5 or 10 μM Aβ(1–42) for 1 h on unstimulated cells had no significant effect on the basal level of P-CREB (as a percentage of control the values after exposure to 5 or 10 μM Aβ(1–42) 115 ± 33 and 120 ± 21%) (Fig. 1C).

The main effect of Aβ(1–42) on the depolarization-induced elevation of P-CREB occurred within the 1st h, and in cultures exposed to high K⁺ for 15 min the P-CREB level was, as a percentage of that in the untreated controls, 50 ± 3.0 or 40 ± 2.0% after treatment with 5 μM Aβ(1–42) for 1 or 24 h (Fig. 1D; n = 3). In contrast, the effect of Aβ(1–42) with random sequence (Aβ(1–42)(R)) failed to influence the high K⁺-induced increase of P-CREB levels (Fig. 1E). After a 1-h treatment with...
Fig. 1. Treatment with Aβ-(1–42) decreased high K⁺-induced CREB phosphorylation in cultured cortical neurons. CREB activation was examined using an antibody against Ser-133-phosphorylated CREB (P-CREB). A, Western blot analysis of CREB. In comparison with control, the amount of P-CREB increased by exposing cortical neuronal cultures to 30 mM KCl for 15 min. Pretreatment with sublethal concentrations of Aβ-(1–42) (5 or 10 μM) for 1 h resulted in a decrease in the amount of P-CREB. B, quantification of levels of P-CREB and T-CREB obtained in three independent experiments shows that Aβ-(1–42) treatment resulted in a concentration-dependent suppression of the K⁺-induced elevation of P-CREB content (9, p < 0.05) but had no significant effect on the level of total CREB (C, control; K, 30 mM KCl; A1, A5, and A10, 30 mM KCl after pretreatment with 1, 5, and 10 μM Aβ-(1–42), respectively). C, pretreatment with 5 or 10 μM Aβ-(1–42) for 1 h on unstimulated cells had no significant effect on the basal level of P-CREB. D, pretreatment with 5 μM Aβ-(1–42) for 1 and 24 h decreased the amount of P-CREB in high K⁺-treated cells. E, pretreatment with 10 μM Aβ-(1–42) with random sequence Aβ(R) had no significant influence on the high K⁺-induced increase of P-CREB levels. Similar results were obtained in three independent experiments.

10 μM Aβ-(1–42)(R) the estimates were, as a percentage of those in the untreated controls, 91 ± 6.7% compared with less than 40% observed after exposure to 10 μM Aβ-(1–42) (Fig. 1B). None of the treatments had significant effects on total CREB levels (e.g. Fig. 1B).

We examined further CREB activation using immunocytochemistry and observed that membrane depolarization induced by exposure to elevated K⁺ resulted in almost all cells in a pronounced increase in P-CREB immunoreactivity, which was markedly attenuated by treatment with Aβ-(1–42) (Fig. 2).

Diffusible Aβ-(1–42) Oligomers Decrease Neuronal Activity-induced Phosphorylation of CREB—There is evidence that neurotoxicity in primary cultures is related to the ability of Aβ-(1–42) to form fibrillary assemblies (3, 8, 26). In addition, Aβ may form small diffusible oligomers (referred to as ADDLs, for Aβ-derived diffusible ligands), which are highly neurotoxic (31, 32). In the ADDL preparation used in the present study the formation of large Aβ assemblies was reduced using clusterin, and the large assemblies that did form were removed by centrifugation, according to the procedure of Lambert et al. (32) (see “Experimental Procedures”). Most importantly, ADDLs cause degeneration of neurons at lower concentrations than conventionally assembled preparations (32) and may share properties with soluble Aβ that is present in the AD brain. Also under our experimental conditions, ADDLs compromised neuronal survival at concentrations lower than those needed to effect neurotoxicity with conventional fibrillar Aβ-(1–42) preparations, causing significant cell loss (23%), already at a concentration of 1 μM after 24 h of exposure. Furthermore, at the sublethal concentrations of 100 nM, ADDL elicited a marked suppression of the high K⁺-induced increase in P-CREB content to 62 ± 13.6% (n = 3) of the levels in the untreated controls (Fig. 3).

To determine whether the observed effects were specific to Aβ-(1–42), the influence of Aβ-(25–35), a toxic fragment of Aβ, and Aβ-(1–40) for 1 and 24 h decreased the amount of P-CREB in high K⁺-treated cells. E, pretreatment with 10 μM Aβ-(1–42) with random sequence Aβ(R) had no significant influence on the high K⁺-induced increase of P-CREB levels. Similar results were obtained in three independent experiments.

was examined. This fragment also elicited neuron loss at 25 μM but, importantly, did not interfere with CREB phosphorylation at the sublethal concentration of 10 μM (Fig. 4, A and B). Therefore, both the fibrillar Aβ-(1–42) and the diffusible Aβ-(1–42) oligomers (ADDLs) interfere at sublethal concentrations...
with neuronal activity-induced signal transduction via CREB, whereas the potent toxic fragment Aβ-(25–35) is inactive.

Sublethal Concentrations of Aβ-(1–42) Decrease NMDA Receptor-mediated CREB Activation—CREB phosphorylation can also be elicited by nerve cell activation through the stimulation of NMDA receptors that play an important role in neuronal plasticity, including certain types of LTP (35, 36). A short exposure to NMDA in our cultures also evoked a marked increase in P-CREB levels, and this effect was suppressed by sublethal concentrations of Aβ-(1–42) (Fig. 5). As a percentage of P-CREB levels in the NMDA-treated cultures, pretreatment with 5 μM Aβ-(1–42) reduced P-CREB levels to 59 ± 7.2% (n = 3). None of the treatments had significant effects on total CREB levels (Fig. 5).

Caspase Activation Does Not Contribute to the Aβ-(1–42)-mediated Decrease in the Activity-induced Phosphorylation of CREB—Although neurons remained viable in the presence of 1–10 μM Aβ-(1–42) during the experimental period, sublethal Aβ-(1–42) may have triggered early apoptotic events that affect CREB signaling. Previous studies suggested that caspase activation plays an important role in Aβ-(1–42)-induced apoptosis in neurons (37, 38). We therefore blocked caspase activities, using the general inhibitor Z-VAD-fmk (39). The cultures in the presence and absence of Aβ-(1–42) were exposed to Z-VAD-fmk under the conditions (150 μM, 1 h of preincubation) when this compound is known to elicit effective caspase inhibition in cortical neurons and PC12 cells (40, 41). The caspase inhibitor had no significant effect on either the basal or K+-induced phosphorylation of CREB and did not influence the Aβ-(1–42)-induced suppression of high K+-activated CREB phosphorylation (Fig. 6). Thus sublethal Aβ-(1–42) suppresses CREB signaling through a mechanism(s) independent of caspase activation.

Low Concentrations of Aβ-(1–42) Decrease CRE Transcriptional Activity and the Induction of BDNF Exon III—To examine the consequences of the action of sublethal concentrations of Aβ on CREB-mediated signal transduction, we analyzed the expression of one of the target genes of CREB, BDNF, which plays a critical role in neuronal survival, differentiation, and plasticity (20). Previous studies showed that the BDNF gene gives rise to four primary transcripts through alternative splicing (42) and that the exon III containing mRNA is the most responsive to neuronal activity in the cerebral cortex and the hippocampus (17, 18). The effect is mediated largely by Ca2+ influx, and the promoter of exon III in the BDNF gene contains a CREB-response element (CRE) (17, 18). As an example of an important CREB-regulated gene, we examined the modulation by Aβ-(1–42) of the activity-dependent expression of exon III-containing BDNF (exon III BDNF). The activation of BDNF exon III promoter was monitored using a transient transcription activity assay with luciferase as a reporter gene. Fig. 7A shows that K+-induced membrane depolarization increased exon III BDNF promoter activity 3-fold and Aβ-(1–42) treatment ameliorated the induction by 31%. We further examined the effect of Aβ-(1–42) treatment on the expression of exon III BDNF, employing a quantitative RT-PCR as described previously (18). Fig. 7B and C, shows that exposure of cells to high K+ increased the expression of exon III BDNF by over 3-fold. Treatment with Aβ-(1–42) had no effect on the basal amount of mRNA (not shown) but decreased the membrane depolarization–elicited elevation of exon III BDNF expression to 38% of the control level.

DISCUSSION

These observations provide the first evidence that sublethal levels of Aβ-(1–42) interfere with gene expression regulated by activity-dependent signal transduction. Treatment of cortical neurons with concentrations of Aβ-(1–42), which did not affect viability and had no apparent influence on the morphology of the cells, resulted in a marked suppression of activity-dependent stimulation of CREB and CREB/CRE-mediated gene tran-

FIG. 5. Treatment with Aβ-(1–42) decreased NMDA-induced CREB phosphorylation in cultured cortical neurons. A, Western blot analysis of P-CREB. Pretreatment with 5 μM Aβ-(1–42) for 1 h attenuated the increase in P-CREB content evoked by exposure to 10 μM NMDA for 10 min. Similar results were obtained in three independent experiments. B, quantification of the effect of pretreatment with 5 μM Aβ-(1–42) for 1 h; estimates are expressed in terms of P-CREB levels obtained in the NMDA-exposed cultures and they are mean ± S.E. from three independent experiments. The effect of Aβ-(1–42) was significant (*, p < 0.05).
mRNA expression, assessed here by evaluating the expression of one of the CREB target genes, BDNF. Because of the critical role of CREB and BDNF in synaptic plasticity (13–15, 20), these observations suggest that low levels of Aβ-(1–42) can engender a dysfunctional encoding state in neurons and may initiate early losses in cognitive function and/or contribute to the propagation of the cognitive deficit in later stage events of AD as Aβ continues to accumulate. In either case, the findings suggest that mechanisms other than neurodegeneration are contributing to losses in brain function.

Previous studies (e.g. Refs. 1, 8, and 34), including from our own laboratory, have established that high levels of Aβ-(1–42) result in neuronal cell death via apoptosis and secondary necrosis. Recent studies (9–11) on transgenic mice overexpressing FAD mutant APPs (1, 4, 5) and observations on AD subjects are consistent with the view that cognitive decline might occur prior to pronounced accumulation of Aβ and before widespread neuronal degeneration takes place in the brain. Animals overexpressing the FAD mutant APP develop progressively histopathological abnormalities including typical neuritic amyloid plaques and dystrophic neurites, but loss of neurons is not a consistent feature and may relate to the genetic background of the animals rather than to the phenotype (4, 7, 43). The mice show functional deficits affecting synaptic plasticity, and the performance of the aged transgenics in spatial working memory tasks is impaired (4). In view of our findings, it is particularly important that in transgenic animals, which were generated in order to increase Aβ levels in the context of relatively low APP expression (Swedish/Indiana double mutations in APP), deficit in long term potentiation was already observed at a relatively young age when amyloid plaques are not yet apparent but an increase in Aβ expression is detectable (5).

Recent observations have shown that soluble intermediate forms of Aβ assemblies, such as Aβ protofilaments (44) and ADDLs (32), can interfere with neuronal function and survival irrespective of the formation of mature Aβ fibrils. Our findings are in agreement with these results. ADDLs compromised neuron survival, and at sublethal levels reduced the activity-dependent signaling via CREB at lower concentrations than fully fibrillar Aβ, consistent with a role of the peptide in disrupting neuronal function during the development of AD.

That low levels of Aβ-(1–42) may compromise brain functions through mechanisms unrelated to degenerative changes was supported by our observation that Aβ-(25–35) at sublethal concentration did not interfere with activity-dependent signal transduction and that caspase inhibitors, which are known to prevent Aβ-(1–42)-induced apoptosis, failed to counteract the Aβ-(1–42)-evoked suppression of CREB activation.

CREB/CRE is known to play a central and highly conserved role in the molecular mechanisms underlying synaptic plasticity, including learning and memory (13–15). As cognitive deficit is central to the pathophysiology of AD, it is relevant that a reduction of P-CREB levels has been observed in the postmortem AD brain (45). Our observations that neuronal activity-induced CREB phosphorylation is suppressed by sublethal levels of Aβ-(1–42) are consistent with these findings. It has been reported, however, that in certain non-neuronal cells Aβ can increase CREB phosphorylation. Thus in undifferentiated PC12 cells Aβ-(1–40) can lead to CREB activation (46), and in cultures of primary microglia and THP1 monocytes, relatively high concentration of fibrillar Aβ-(25–35) (50 μM) has a similar effect (47). Responses of these cells to Aβ are markedly different from those observed in nerve cells. In contrast to these non-neuronal cells, our data show that Aβ-(1–42) had no significant effect on CREB phosphorylation in resting neurons. Furthermore, in contrast to Aβ-(1–42), Aβ-(25–35) had no significant influence on high K+–induced activation of CREB. The careful study of McDonald et al. (47) elucidated that the signal transduction mechanisms leading to 50 μM Aβ-(25–35)-induced CREB phosphorylation in microglia and monocytes involve protein tyrosine kinase-dependent activation of two parallel pathways, extracellular signal-regulated kinase and p38 mitogen-activated protein kinases. On the other hand, in nerve cells the depolarization-induced phosphorylation of CREB is primarily dependent on Ca2+- influx-mediated activation of Ca2+-calmodulin-dependent protein kinase IV. This has been demonstrated.

Sublethal β-Amyloid Reduces Activity-dependent CREB Signaling

![Fig. 6. Z-VAD-fmk did not influence the effect of Aβ-(1–42) in reducing the high K+–induced elevation of the P-CREB content.](http://www.jbc.org/)

Cultured cortical neurons at 5 DIV were exposed to 10 μM Aβ-(1–42) in the presence or absence of 150 μM Z-VAD-fmk for 1 h, before treatment with 30 mM KCl for 15 min. The experiment was repeated with a similar outcome; Z-VAD-fmk could not prevent the Aβ-(1–42)-induced suppression of the neuronal activity-evoked elevation of P-CREB levels.

![Fig. 7. Effect of Aβ-(1–42) on BDNF exon III expression in cultured cortical neurons.](http://www.jbc.org/)

A, activity of the BDNF exon III promoter measured by the luciferase assay. Cortical neurons at 2 DIV were transfected with plasmid pH2170Luc containing the promoter of BDNF exon III comprising the CRE-like sequence and exon III fused to a luciferase reporter gene. After 40 h, cultures were switched to fresh medium and incubated for 1 h in the presence or absence of 5 μM Aβ-(1–42), before the addition of a solution of KCl (final concentration 30 mM) or vehicle and further incubation for 9 h. Transcription activity was assayed by measuring luciferase activity and expressed as the ratio of the luciferase activity in extracts from cells exposed to high K+ to the luciferase activity in extracts from unstimulated cells. High K+–elicited the activation of BDNF exon III promoter (K+). Aβ-(1–42) treatment suppressed the high K+–induced activation of BDNF exon III promoter (K+–A). Each value represents the mean ± S.D. of two independent experiments. B, the expression of BDNF exon III gene and GAPDH gene (for normalization of the total RNA in the samples) was measured by RT-PCR. Total RNA (100 ng) was reverse-transcribed into single-stranded cDNA, and the cDNA was amplified by PCR with exon-specific primers. The PCR products were separated by electrophoresis on 6% polyacrylamide gels. C, quantification of RT-PCR products by PhosphorImager. The amount of total RNA in the samples was normalized to the amount of GAPDH. Each value represents the mean ± S.E. from three independent experiments. Exon III BDNF mRNA levels were significantly increased in the high K+–exposed cells; pretreatment with 5 μM Aβ-(1–42) significantly attenuated the high K+–induced elevation of exon III BDNF mRNA levels (p < 0.05).
previously (17) and confirmed in our studies by the pronounced inhibition of the high K⁺-induced CREB phosphorylation by the Ca²⁺-calmodulin-dependent protein kinase inhibitor KN62 (not shown).

The transfection of three familial APP mutations (V642I, V642F, and V642G) in neurons resulted in suppressed transcriptional activity of CRE (48). Interference with events downstream of activated CREB was supported by the finding that sublethal Aβ(1–42) suppressed the neuronal activity-dependent transcription of the exon III-containing BDNF. BDNF is a complex gene that contains five exons (42). The coding region is entirely in exon V, whereas each of the first four exons has a unique promoter, containing different regulatory elements on the 5′-flanking region. Each of the four 5′ exons is, therefore, differentially regulated and gives rise to four primary BDNF transcripts. The promoter of exon III contains a CRE and a response element to a novel Ca²⁺-regulated factor (17, 18); thus BDNF is a CREB target gene. The relevance of sublethal Aβ(1–42)-induced suppression of the neuronal activity-dependent phosphorylation of CREB and the induction of the CREB target gene BDNF is underlined by the observation that the content of both the BDNF transcript and protein is markedly reduced in brain regions, such as the hippocampus, which are severely affected in AD (23–25). Because of the role of CREB and BDNF in neuronal plasticity, including learning and memory (20, 21), the reduction may contribute to the cognitive deficit in AD. Although the BDNF protein is not the exclusive product of the exon III-containing transcript, our finding that sublethal levels of Aβ(1–42) result in the suppression of the activity-dependent phosphorylation of CREB and the consequent expression of BDNF indicates that the peptide may interfere with neuronal functions early in the course of the disease, preceding the stage when massive neuronal degeneration is evident.

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