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Alzheimer amyloid-β peptides block the activation of C/EBPβ and C/EBPδ in glial cells

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ABSTRACT

Members of the CCAAT/enhancer binding protein (C/EBP) family of transcription factors have been reported to be up-regulated in Alzheimer's disease. In the present study, we have investigated the effects of amyloid-β (Aβ) peptides on C/EBPβ and C/EBPδ, previously shown to be induced by inflammatory stimuli in glial cells. Surprisingly, electrophoretic mobility shift assay showed that both Aβ(1–42) and Aβ(25–35) blocked C/EBP activation induced by the inflammatory cytokine interleukin-1β (IL-1β) or lipopolysaccharide (LPS) in mixed primary glial cell cultures from rat. Aβ also blocked IL-1β- or LPS-induced C/EBP protein levels. The most prominent effects were observed on DNA binding activity and protein levels of C/EBPδ. Our results demonstrate a dysregulation of C/EBP when glial cells are activated in the presence of Alzheimer Aβ peptides.

The family of CCAAT/enhancer binding protein (C/EBP) leucin zipper transcription factors have six members; C/EBPα-ζ. C/EBPs most commonly form homo- or heterodimers within their family but also with other leucine- and non-leucine zippers. Target genes are typically involved in differentiation, proliferation, metabolism, and inflammation. C/EBP has primarily been studied in the liver, but is also expressed in various other tissues, including brain [1–3]. In recent years, C/EBPβ and C/EBPδ have been suggested to be involved in neuroinflammation [3–9].

Neuroinflammation plays a role in several neurodegenerative disorders including Alzheimer's disease (AD) [10–13]. In AD, it is believed that accumulation of amyloid-β (Aβ) peptides and formation of plaques, activate glial cells and trigger a chronic inflammatory response. Several transcription factors have been shown to be involved in glial activation, including nuclear factor-κB (NF-κB) and C/EBP [5,7–9]. Interestingly, C/EBP family members have also been found to be up-regulated in AD [3,4,6,14].

The aim of this study was to investigate whether Alzheimer Aβ peptides had any effects on C/EBPβ and C/EBPδ in activated glial cultures.

Materials and methods

Cell cultures and treatments. Primary mixed glial cultures (90–95% astrocytes and 5–10% microglial cells) were prepared from Sprague–Dawley pups, and maintained as previously described [15]. Cells were treated with 10 μM Aβ(1–42) (American Peptide), 100 μM Aβ(25–35) or Aβ(35–25) (Bachem), 10 ng/ml rat recombinant IL-1β (Biosource), and/or 1 μg/ml lipopolysaccharide (LPS) (Sigma) for 3 h and then analyzed by electrophoretic mobility shift assay (EMSA) or Western Blot. Aβ(1–42) was prepared yielding an oligomeric population [16,17]. Oligomer formation was analyzed by Western blot [16–18], (see supplementary data). Aβ(25–35) and Aβ(35–25) were prepared as previously described [15]. The Aβ(25–35) preparation was determined to consist of approx. 30% fibrils by Congo Red staining. The experimental procedures were performed in accordance with international standards on animal welfare and were approved by Stockholm North Local Committee on Ethics of Animal Experiments.

Electrophoretic mobility shift assay (EMSA). Preparation of nuclear extracts and EMSA was performed as previously described [15] with minor modifications. The oligonucleotides used were: wild-type C/EBP sense oligonucleotide 5′-TGCAGA TTGCGCAATCTGCA-3′, mutated C/EBP sense oligonucleotide 5′-TGCAGAgaGtagtc TCTGCA-3′ (mutated nucleotides in lower-case letters), wild-type NF-κB sense oligonucleotide 5′-AGTTGAGGGGACTTTCCCAGGC-3′ (MWG). Subunit specific antibodies were used for supershift analysis to identify C/EBPβ and C/EBPδ (sc-150X and sc-151X, respectively, Santa Cruz Biotechnology). For supershift analysis, samples were incubated with the antibody of interest for 30 min prior to the addition of the radiolabeled probe. To quantify the supershifted samples, the intensity of the remaining band was determined, and subtracted from the intensity of the band derived from the corresponding nuclear extract not exposed to the antibody.

Western blot assay. Preparation of cell lysates and Western blot was performed as previously described [19] with minor modifications. Fifteen micrograms of protein was separated on a 12% polyacrylamide gel. Primary antibody concentrations were as follows: C/EBPβ 1:4000, C/EBPδ 1:1000 (sc-150X and sc-151X respectively, rabbit polyclonal, Santa Cruz Biotechnology) and α-tubulin 1:15000 (T-9026, mouse monoclonal, Sigma). Horseradish peroxidase-coupled anti-rabbit or anti-mouse antibodies were used at a dilution of 1:2000.

Statistical analysis. Statistical significances were determined by one-way ANOVA followed by Tukey's or Bonferroni's post tests.

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Results

C/EBP binding activity is blocked by Aβ peptides in activated glial cells

In order to study the binding activity of C/EBP, primary mixed glial cells were stimulated with IL-1β or LPS for 3 h alone or in the presence of different Aβ peptides and analyzed with EMSA. Treatment with IL-1β or LPS resulted in an increased activation of C/EBP binding activity to approx. 180% and 210%, respectively, as compared to control (Fig. 1A and B). Surprisingly, Aβ(1–42) and Aβ(25–35) significantly blocked IL-1β- and/or LPS-induced C/EBP binding activity, whereas no effect could be observed using the control peptide Aβ(35–25) (Fig. 1A and B). Aβ(1–42) was dissolved in a small volume of DMSO. No effects of this concentration (0.2%) could be observed (data not shown). The C/EBP EMSA showed three bands (Fig. 1C). A 20- or 100-fold excess of unlabeled C/EBP probe or mutated C/EBP probe confirmed that the upper band corresponds to specific binding whereas the lower bands correspond to non-specific binding. In addition, a 20- or 100-fold excess of unlabeled NF-κB probe did not affect the specific band, demonstrating that the protein complexes selectively bind to the C/EBP consensus sequence.

C/EBPδ binding activity and protein levels are blocked by Aβ in activated glial cells

Next, the effects of Aβ on C/EBPδ were investigated. EMSA showed that IL-1β and LPS significantly induced binding activity

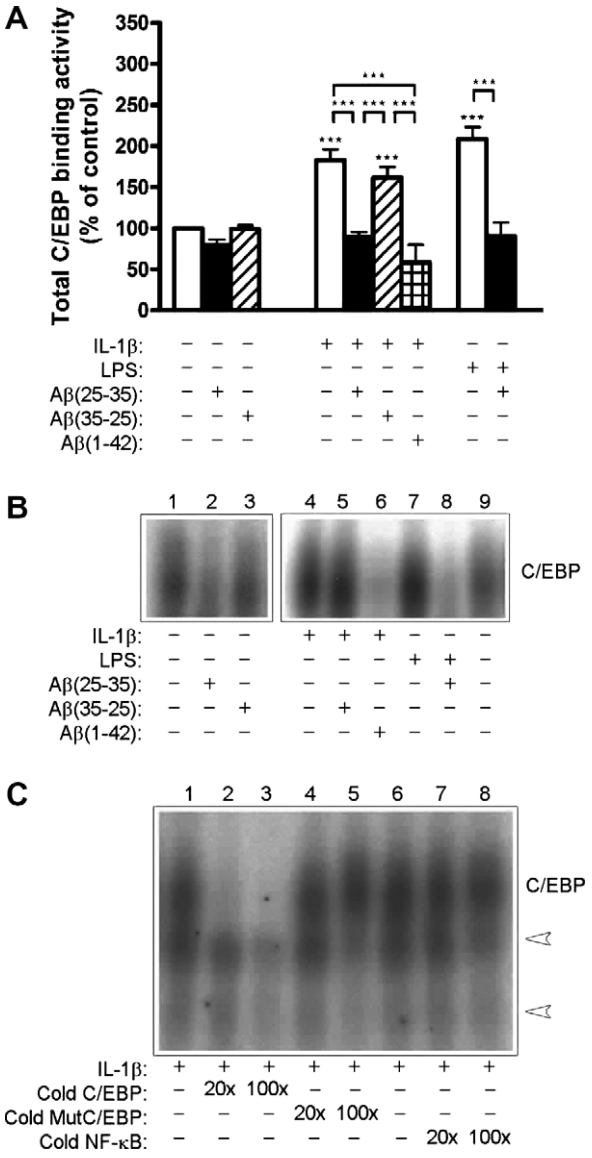

Fig. 1. IL-1β- or LPS-induced C/EBP binding activity is blocked by Aβ. Glial cells were treated for 3 h as indicated in figure. Total C/EBP binding activity was analyzed by EMSA. (A) C/EBP binding activity expressed as % of control. Significance level: $^{***}P < 0.001$ compared to control if not otherwise indicated, means ± SEM, $N = 6–15$. (B) Representative EMSA showing the effects on C/EBP binding activity. (C) EMSA indicating specific/non-specific binding. Cells were treated with IL-1β. A 20- or 100-fold excess of unlabeled C/EBP probe (cold C/EBP), unlabeled mutated C/EBP probe (cold MutC/EBP) or unlabeled NF-κB probe (cold NF-κB) in the annealing reaction confirmed that the upper band corresponds to specific binding whereas the lower bands correspond to non-specific binding (arrowheads).

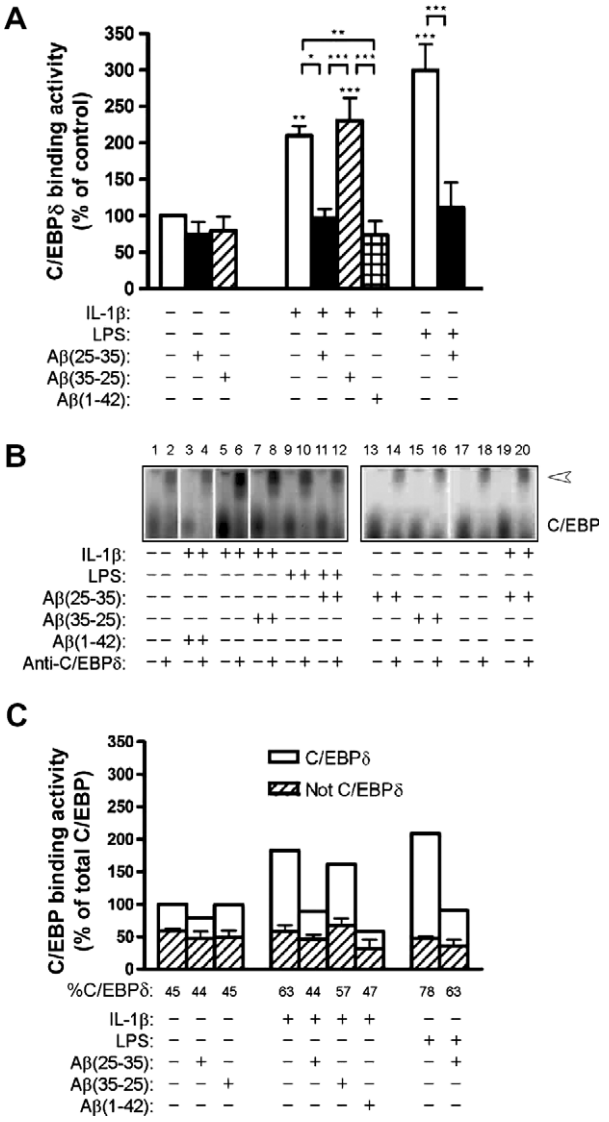

Fig. 2. IL-1β- or LPS-induced C/EBPδ binding activity is blocked by Aβ. Cells were treated for 3 h as indicated in figure. C/EBPδ binding activity was analyzed by EMSA. (A) C/EBPδ binding activity expressed as % of control. Significance levels: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared to control if not otherwise indicated, means ± SEM, $N = 4–10$. (B) Representative EMSA showing total C/EBP binding and supershifts caused by anti-C/EBPδ. Supershifts of complexes containing C/EBPδ are indicated with an arrowhead. (C) Binding activity of complexes not containing C/EBPδ (hatched) and complexes containing C/EBPδ (open). Below each bar the % C/EBPδ of total C/EBP binding activity is indicated. There were no statistically significant differences between the groups of C/EBP binding complexes not containing C/EBPδ (cf., statistical significances indicated for total C/EBP binding activity in Fig. 1A and on C/EBPδ in Fig. 2A).