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# Interleukin-1β dissociates β-amyloid precursor protein and β-amyloid peptide secretion

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**Abstract** A heightened production of interleukin 1β (IL-1β) has been reported in microglial-associated amyloid deposits in Alzheimer's disease (AD) brains. These plaques are composed predominantly of β/A4 peptide derived from β-amyloid precursor protein (βAPP). We demonstrate that short-term (1 h) IL-1β-treatment increases $\beta APP_s$ secretion and concomitantly decreases cell-associated βAPP in human H4 neuroglioma cells. Long-term (5 h) IL-1β treatment did not alter secreted or cell-associated βAPP content. In contrast, the secretion of β/A4-containing epitope was not affected by short-term IL-1β stimulation; however, long-term IL-1β treatment decreased the amount of β/A4-containing epitope secreted from the cells. These results show that IL-1β modifies the processing and secretion of βAPP to exacerbate perhaps the neuropathology of AD.

*Key words:* β-Amyloid precursor protein; β-Amyloid peptide; Interleukin 1β; H4 neuroglioma

## 1. Introduction

Alzheimer's disease (AD) pathology is characterized by lesions in brain regions important for intellectual function. The characteristic lesions of AD as well as Down's syndrome are amyloid depositions in the brain parenchyma in selected regions and in its vasculature. The amyloid deposits are composed predominantly of β/A4 peptide [1], derived from the transmembrane regions of β-amyloid precursor protein (βAPP) by proteolytic cleavage [2]. The increased production of β/A4 in some individuals leads to early onset of AD [3,4], implicating β/A4 peptide as a pathogenic factor in AD.

Proinflammatory cytokines may also be involved in AD [5,6]. For example, AD and Down's syndrome brains contain 30-fold more interleukin-1-producing microglia and astrocytes as compared to age-matched controls [7]. At the transcriptional level, interleukin 1β (IL-1β) increases βAPP mRNA in primary cultures of astrocytes, cortical neurons and endothelial cells [5,8], but not in oligodendrocyte-derived cell lines [9]. These differences in interleukin-1 effects may reflect differences among cell lines. At the protein level, short-term IL-1β-stimulation of glial and endothelial cell lines increases secreted βAPP ($\beta APP_s$) [10] but appears to have little affect on β/A4 secretion [11].

In the present study, we examine the effect of IL-1β stimulation on the regulation of βAPP and β/A4-containing epitope secretion as well as cell-associated βAPP in a neuroglioma cell line. Our results suggest that short-term IL-1β stimulation alters both secreted and cell-associated βAPP but does not affect the secretion of β/A4-containing epitope. However, long-term IL-1β stimulation does not alter secreted or cell-associated βAPP but decreases the secretion of β/A4-containing epitope by about 50%.

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## 2. Materials and methods

### 2.1. Cell culture

Human neuroglioma H4 cells (ATCC), producing high amounts of β/A4 peptide [12], were cultured in DMEM (Gibco-BRL) containing 10% FCS (Hyclone) in 100 mm tissue culture dishes (Falcon) at $2 \times 10^6$ cells/10 ml. After 48 h, the medium was discarded, and the cells were washed 3 times with Puck's Saline (Gibco-BRL). The cells were then cultured in 4 ml of OptiMEM I Reduced Serum Medium (Gibco-BRL) and supplemented with recombinant human IL-1β (Genzyme) for 1 or 5 h at 37°C, 5% $CO_2$ or left untreated. The conditioned medium was collected, supplemented with protease inhibitors [13], frozen with dry ice and stored at −20°C. Cellular protein extracts were obtained after washing the cells 3 times in Puck's Saline and lysing the cells in 0.7 ml lysis buffer (20 mM Tris, pH 8.6, 1% Triton X-100) containing protease inhibitors [13]. The lysates were frozen with dry ice and stored at −20°C.

### 2.2. Antibodies

Murine monoclonal antibodies (MAb) 22C11, 6E10 and 4G8 have been described previously [14–16]. MAb 22C11 recognizes the amino terminus of $\beta APP_s$; MAb 6E10 recognizes the carboxy terminus of $\beta APP_s$ and the amino terminus of β/A4 peptide (residues 1–16); and MAb 4G8 recognizes the mid-region of β/A4 peptide (residues 17–24) but not $\beta APP_s$. Protein G purified MAb 6E10 was biotinylated with NHS-LC-biotin (Pierce).

### 2.3. SDS-PAGE and Western blot analysis

Secreted and cell-associated βAPP were detected by Western blot. H4 conditioned medium was diluted 1:2 in SDS-PAGE reducing sample buffer and heated for 5 min at 95°C. Equal volumes were loaded onto 4–12% Tris-glycine gels (Novex). Detergent-extracted cellular proteins were quantitated by the BCA protein assay (Pierce Chemical Co.). Equal amounts of protein (4 μg/15 μl) were loaded per lane on 4–12% gels. Electrophoresis was performed at 100 V, and proteins were transferred to nitrocellulose (Hybond-ECL, Amersham) [17]. The blots were blocked with 5% non-fat dry milk (Carnation) and then incubated with anti-βAPP MAb (22C11, 5 μg/ml). After washing, the blots were incubated with sheep anti-mouse Ig-HRP (Amersham, 1:1500). All washes and dilutions of non-fat dry milk and antibodies were with TBST (20 mM Tris, pH 8.0, 0.5 M NaCl, 0.1% Tween 20) for 1 h. Immunoreactivity was detected using Enhanced Chemiluminescence on X-OMAT LS film according to the manufacturer's recommendations (ECL detection reagents, Amersham). The entire procedure was performed at room temperature. The films were scanned using optical reflectance (Visage Electrophoresis Gel Analysis System, Version 4.6, Millipore),


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and the integrated optical density (IOD) of each band was calculated. The data were analyzed by the Student's *t*-test (Statview 512). All blots contained an internal untreated control to which treated samples were compared. The results are reported as βAPP relative units, which is the ratio of IL-1β-treatment IOD/untreated control IOD.

### 2.4. ELISA

β/A4-containing epitope was detected using a modified double antibody capture immunoassay [14]. The ELISA is specific for amino acids 1–28 of the β/A4 peptide but does not detect secreted βAPP. ELISA plates (MaxiSorb, Costar) were coated with 0.5 μg of MAb 4G8 in 10 mM sodium carbonate, pH 9.6 overnight at 4°C and then blocked with 1% BSA and 0.1% bovine γ-globulin in TBST. Samples (freshly collected conditioned medium) and standards (β/A4 peptide, residues 1–40) were added to each well and incubated overnight at 4°C. The plates were washed and incubated with 50 μl of biotinylated MAb 6E10 (3 μg/ml in TBST, 1% BSA) for 1 h, washed, then incubated for an additional hour with 50 μl of avidin-alkaline phosphatase conjugate (1:5000 dilution into TBST, 1% BSA). The plates were washed 3 times with TBST before adding 100 μl of Attophos (JBL Scientific, Inc.) to each well. Fluorescent intensity was read using a Cytofluor 2350 multiwell fluorescence plate reader (Millipore, 440 nm excitation filter, bandwidth 20 nm; 560 nm emission filter, bandwidth 20 nm; sensitivity 2). For detecting low levels of β/A4 (< 50 pg/ml), the plates were incubated overnight in the dark before analysis. The entire procedure except overnight incubations was performed at room temperature.

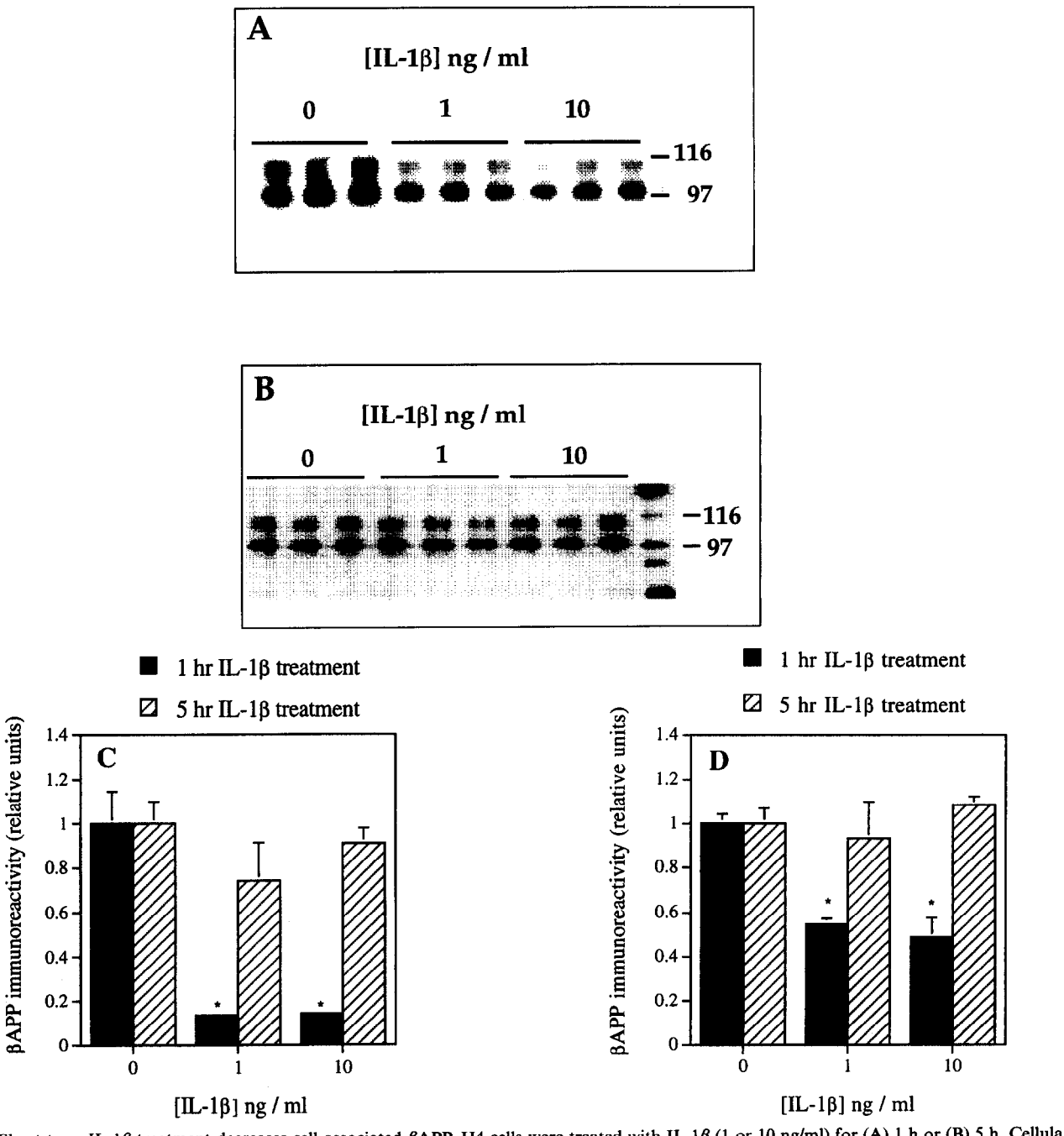
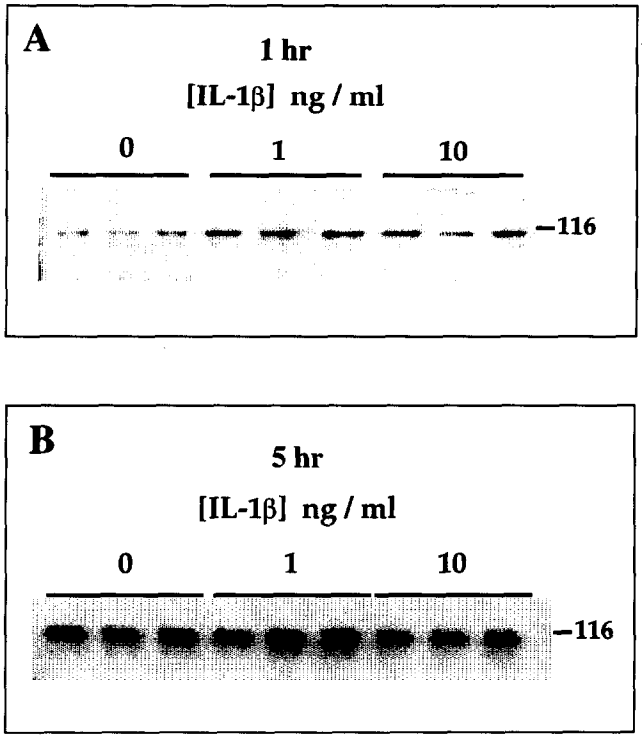



Fig. 1. Short-term IL-1β treatment decreases cell-associated βAPP. H4 cells were treated with IL-1β (1 or 10 ng/ml) for (A) 1 h or (B) 5 h. Cellular protein was collected from separate dishes by detergent lysis, and 4 μg of each sample (soluble fraction) was loaded onto 4–12% gradient gels. After electrophoresis and transfer to nitrocellulose, the blot was probed with MAb 22C11 and MAb 6E10 and developed with ECL. (C) 113 kDa and (D) 97 kDa immunoreactive bands were quantified by optical reflectance. $^*P < 0.05$ compared to the untreated 1 h control, $n = 3$.

## 3. Results

Cell-associated βAPP content was determined by Western blot analysis of detergent-extracted cellular proteins from H4 cells. Immunoreactivity was measured using either MAb 22C11 or MAb 6E10. MAb 22C11 detects both βAPP and the amyloid precursor-like protein (APLP); 6E10 detects βAPP but does not detect APLP which lacks β/A4 epitopes. In all samples, two major bands of 113 and 97 kDa were detected suggesting APLP was not a major contributor to the immunoreactivity measured (Fig. 1A,B). Smaller proteins were also detected, presumably representing partially translated or partially degraded βAPP (data not shown).



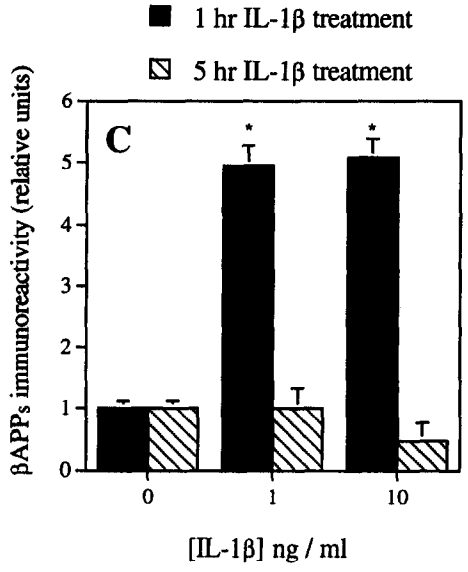




Fig. 2. Short-term IL-1β treatment enhances $\beta APP_s$. H4 cells were treated with IL-1β (1 or 10 ng/ml) for (A) 1 h or (B) 5 h. The conditioned medium from separate dishes was loaded onto 4–12% gradient gels. After electrophoresis and transfer to nitrocellulose, the blots were probed with MAb 22C11 and MAb 6E10 and developed with ECL. (C) $\beta APP_s$ immunoreactive bands were quantified by optical reflectance. The results are representative of 4 experiments. $^*P < 0.05$ compared to the untreated 1 h control, $n = 3$.

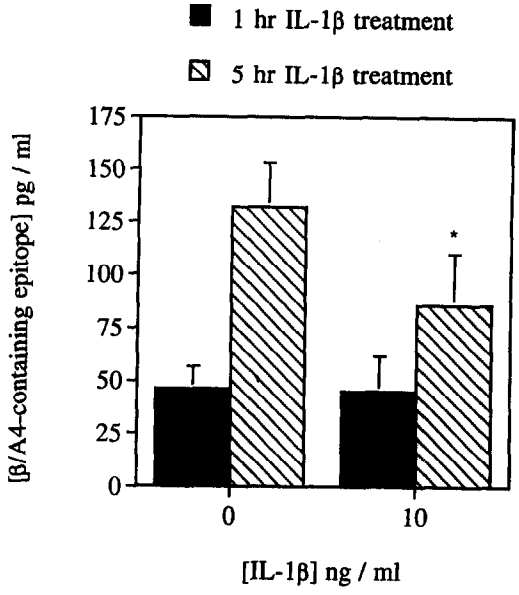



Fig. 3. Short-term IL-1β treatment does not affect β/A4 secretion. Conditioned medium from IL-1β-treated and untreated H4 cells was assayed by ELISA for β/A4-containing epitopes after 1 h or 5 h of cytokine treatment. The results are representative of 4 experiments. $^*P < 0.05$ compared to the untreated 5 h control, $n = 3$.

Short-term (1 hour) IL-1β treatment reduced the amount of the 113 kDa cell-associated form of βAPP in H4 cells as compared to untreated cells at 1 h (Fig. 1C). After long-term (5 h) IL-1β treatment, H4 cells contained about the same amount of 113 kDa cell-associated βAPP as the untreated cells at 5 h. Short-term IL-1β treatment reduced the content of the 97 kDa cell-associated form approximately 2-fold as compared to untreated 1 hour control cells (Fig. 1D). After 5 h of IL-1β treatment, the amount of the 97 kDa form was approximately the same as the untreated 5 h control cells.

Culture medium was collected from IL-1β-stimulated and unstimulated H4 cells to determine the effect of IL-1β on $\beta APP_s$. IL-1β [1–10 ng/ml] induced a maximal change after 1 h in $\beta APP_s$ as detected on Western blots (Fig. 2). All blots showed a band of immunoreactivity having an apparent size of ~113 kDa. The scanned blots show that H4 cells stimulated with IL-1β for 1 h secreted approximately 5-fold more $\beta APP_s$ than the unstimulated 1 h control cells (Fig. 2C). The actual amount of $\beta APP_s$ secreted into the medium over 5 h from untreated cells was about 8-fold greater than $\beta APP_s$ content in the medium from untreated cells at 1 h (data not shown). In contrast, after 5 h of IL-1β treatment, the content of $\beta APP_s$ in the medium was the same as that secreted from the corresponding untreated control cells (Fig. 2B,C). It appears after the initial burst of βAPP secretion at 1 h, IL-1β treatment reduces the apparent rate at which βAPP is secreted from the cells.

β/A4-containing epitope was measured in H4 conditioned medium by ELISA. H4 cells treated with IL-1β for 1 h produced the same amount of β/A4-containing epitope as un-

treated cells cultured for 1 h (Fig. 3). H4 cells treated with IL-1$\beta$ for 5 h produced approximately half of the amount of $\beta$/A4-containing epitope as unstimulated control cells cultured for 5 h.

## 4. Discussion

The increase measured in $\beta APP_s$ after stimulation with IL-1$\beta$ for 1 h (short-term) indicates that this proinflammatory cytokine enhances secretion of $\beta$APP. The secretion seems transcriptionally-independent since the transcription of the $\beta$APP gene (> 400 kB) and the subsequent translation of the message (100–120 kDa) require more than 1 h as suggested by others [18,19]. Our results are consistent with those of Buxbaum et al. who attributed enhanced secretion of $\beta$APP by IL-1$\beta$ to metabolic changes rather than to changes in transcriptional rate [10]. We do not believe IL-1$\beta$ induced aberrant processing of $\beta$APP. Immunoreactive $\beta APP_s$ was detected on blots probed with MAb 6E10 but not MAb 4G8 (data not shown). Thus, IL-1$\beta$ appears to cause normal $\alpha$-secretase cleavage of cell-associated $\beta$APP to give rise to $\beta APP_s$.

Short-term IL-1$\beta$ treatment did not affect the quantity of $\beta$/A4-containing epitope secreted into the medium. This confirms the observation by Buxbaum et al. [11] that IL-1$\beta$-induced $\beta APP_s$ and $\beta$/A4-containing epitope secretion are not reciprocally linked. This result is in marked contrast to the effects of muscarinic agonists and phorbol esters on $\beta$APP and $\beta$/A4 secretion [20–22]. Thus, the fate of $\beta$APP may depend on the second messengers activated by different receptor–ligand interactions. The nature of these signal transduction systems induced by IL-1$\beta$ are being explored.

Our results suggest that the secretion of $\beta$/A4-containing epitope and $\beta APP_s$ are not reciprocally related. Their secretory processes may be differentially affected by IL-1$\beta$ stimulation, and short-term IL-1$\beta$ stimulation may have long-term effects on $\beta$APP processing that are not initially reflected in $\beta$APP secretion. This implies that more complicated metabolic processing of $\beta$APP may occur under conditions not revealed in earlier studies using carbachol and phorbol esters. Moreover, the experiments should be performed in other cell types to determine if the IL-1$\beta$ effect pertains only to the neuroglioma cells used here.

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