The amylloid precursor protein (APP) has been associated with Alzheimer’s disease (AD) because APP is processed into the β-peptide that accumulates in amyloid plaques, and APP gene mutations can cause early onset AD. Inflammation is also associated with AD as exemplified by increased expression of interleukin-1 (IL-1) in microglia in affected areas of the AD brain. Here we demonstrate that IL-1α and IL-1β increase APP synthesis by up to 6-fold in primary human astrocytes and by 15-fold in human astrocytoma cells without changing the steady-state levels of APP mRNA. A 90-nucleotide sequence in the APP gene 5′-untranslated region (5′-UTR) conferred translational regulation by IL-1α and IL-1β to a chloramphenicol acetyltransferase (CAT) reporter gene. Steady-state levels of transfected APP(5′-UTR)/CAT mRNAs were unchanged, whereas both baseline and IL-1-dependent CAT protein synthesis were increased. This APP mRNA translational enhancer maps from +55 to +144 nucleotides from the 5′-cap site and is homologous to related translational control elements in the 5′-UTR of the light and heavy ferritin genes. Enhanced translation of APP mRNA provides a mechanism by which IL-1 influences the pathogenesis of AD.

The amylloid precursor protein (APP) is a 110–130-kDa type I membrane-spanning glycoprotein expressed ubiquitously in mammalian tissues (1, 2). A portion of APP is processed constitutively into 40–42-amino acid β-amyloid (Aβ) peptides, which then polymerize and deposit as the amyloid filaments as one of the pathological hallmarks of Alzheimer’s disease (AD) and Down syndrome (3–7). The regulation of APP gene expression as a pathogenic factor for AD has received considerable attention. Several putative physiological activators of APP gene transcription have been defined (8–10). Overexpression of APP evidently can cause AD because all individuals with Down syndrome have an extra copy of the APP gene on chromosome 21 and invariably develop AD pathology by the age of 40–50 years (11, 12).

In addition to altered Aβ cleavage, secretion, and deposition (5), accumulating evidence has revealed that local inflammation at the site of developing extracellular plaques in the brain is important to AD pathogenesis (13–15). For example, α1-antichymotrypsin (ACT) is present in amyloid plaques, and its production by adjacent astrocytes suggests the occurrence of an inherent inflammation in the AD brain, similar to the hepatic acute phase response (16, 17). Other significant markers, such as interleukin-1 (IL-1)-positive microglia and complement protein, confirm the presence of local inflammatory events during AD progression (18, 19). Epidemiological studies identifying traumatic head injury as a risk factor for AD strengthen the hypothesis that inflammatory mechanisms contribute to the disease pathogenesis (20). Hippocampal lesion has been shown to increase APP immunoreactivity in neighboring astrocytes (21). In vitro studies, and recently an in vivo study, have shown that certain proteins, e.g. ACT and apolipoprotein E (apoE), which are expressed during traumatic injury and inflammation of the brain parenchyma, might regulate the polymerization of Aβ peptides into amyloid filaments (22–27).

IL-1 is the first proinflammatory cytokine secreted after the activation of macrophage/microglial cells (28). IL-1 is expressed abundantly in microglia around developing amyloid plaques in brain cells, particularly in those brain regions that are prone to develop the mature amyloid plaques enriched in β-sheet protein structure (17, 18, 29). IL-1 action is mediated by two separate cytokines, IL-1α and IL-1β, which share low sequence homology (30%) and are encoded by two separate genes derived from a common ancestor gene (30). IL-1α and IL-1β target the same signaling receptor and exert overlapping proinflammatory effects, although the processing and site of action of these cytokines differ (28).
Microglial IL-1, which is known as a stimulator of astroglial proliferation (31), is increased in the rat brain after injury (32). A protein kinase C-dependent pathway (9) has linked IL-1 to a 3-fold increase in APP gene expression in human endothelial cells at the level of enhanced transcription. IL-1 greatly increased the transcriptional regulation of the amyloid plaque-associated protein ACT in human primary astrocytes (17) and a human astrocytoma cell line (33). However, a smaller induction of APP gene transcription by IL-1 in rat neuronal cells was not matched by an increase in the steady-state levels of APP mRNA in glial cells, including astrocytes (2, 34).

In this report we have identified and characterized a novel mechanism of IL-1-dependent regulation of APP gene expression at the level of increased APP mRNA translation in astrocytes. Here, increased synthesis of APP by IL-1 was found to be mediated through a translational regulatory sequence in the 5'-untranslated region (5'-UTR) of APP mRNAs. We showed previously that IL-1 specifically enhances translation of the mRNAs encoding the light (L) and heavy (H) subunits of ferritin, the central iron storage protein present in all cells (35). The APP mRNA 5'-UTR sequence can fold into a single RNA stem-loop and is related to hepatic RNA enhancers, the acute box (36), and is related to hepatic RNA enhancers, the acute box (36).

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Immunocytochemistry**

Primary human astrocytes were prepared by trypsinization of human fetal brain tissue as described previously (17), treatment with 5 mM H-Leu-O-methyl ester to eliminate microglia (38), seeding onto coated plates, and growing to 70% confluence in DMEM (low glucose) supplemented with 10% fetal bovine serum. For immunohistochemistry the cells were grown on 10 μg/ml poly-L-lysine-coated microscope slides, washed briefly in 1 × PBS, and then fixed in 4% paraformaldehyde and 0.1% Triton X-100 in 1 × PBS, pH 7.4, for 30 min on ice. The cells were then incubated in 10% normal goat serum (Life Technologies, Inc.) and 0.4% Triton X-100 in 1 × PBS for 30 min at 37 °C to block nonspecific binding. The primary antibody (monoclonal anti-glial fibrillary acidic protein (GFAP), clone G-A-5, Sigma, dilution 1:400), was applied in 1% normal goat serum and 0.4% Triton X-100 in 1 × PBS for 1 h at 37 °C. The astrocytes were washed for 5 min in 1 × PBS and incubated with the secondary antibody (affinity-purified polyclonal Cy3-labeled goat anti-mouse IgG (H+L), Jackson ImmunoResearch, dilution 1:400) in 1% normal goat serum and 0.4% Triton X-100 in 1 × PBS for 30 min at room temperature. The cells were again washed in 1 × PBS for 5 min, counterstained with 4 mg/ml 4,6-diamidino-2-phenylindole, mounted in 50% glycerol, and examined with a light microscope ( Axioskop, Zeiss).

The U373MG astrocytoma cell line was obtained from Dr. H. Fine (DFCI, Boston, MA) and cultured on uncoated dishes to 60–80% confluence in DMEM (high glucose) supplemented with 10% fetal bovine serum.

**Plasmid Constructs**

The eukaryote expression vector pSV CAT contains a unique StuI site 45 base pairs (bp) downstream from the SV40 early T-antigen promoter. A unique HindIII site is present 17 bp further downstream from the StuI site (62 bp from the CAT gene 5′-cap site (36)). The pSV

**APP Synthesis**

**Primary Astrocytes—**GFAP-positive astrocytes (1 × 10⁶ cells/well) and astrocytoma cells (70% confluent) were measured for intracellular APP synthesis and ferritin synthesis after stimulation with 0.5 ng/ml recombinant IL-1α (Genzyme), 0.5 ng/ml IL-1β (Genzyme), 10 μM ferrotransferrin (Boehringer Mannheim), 100 μM desferrioxamine (Ciba Geigy), or left untreated as controls. Cell numbers from individual wells were counted to ensure that 1 × 10⁶ cells were present in each well at the beginning of each labeling experiment. Astrocytes were preincubated for 15 min in methionine-free medium (RPMI 1640; Life Technologies, Inc.) and pulse labeled for 30 min with 300 μCi/ml [35S]methionine. Each microtiter plate was washed twice in cold PBS at 4 °C. The amount of incorporation of [35S]methionine into the CAT gene was measured for 25 μl of STEN buffer, 22% Nonidet P-40, 2 mM EDTA, 50 mM Tris, pH 7.6) using a sterile glass rod. After 30 min, the labeled cells were washed briefly in 1 × PBS, pH 7.4, for 30 min on ice. The cells were then incubated in 10% normal goat serum (Life Technologies, Inc.) and 0.4% Triton X-100 in 1 × PBS for 30 min at 37 °C to block nonspecific binding. The primary antibody (monoclonal anti-glial fibrillary acidic protein (GFAP), clone G-A-5, Sigma, dilution 1:400), was applied in 1% normal goat serum and 0.4% Triton X-100 in 1 × PBS for 1 h at 37 °C. The astrocytes were washed for 5 min in 1 × PBS and incubated with the secondary antibody (affinity-purified polyclonal Cy3-labeled goat anti-mouse IgG (H+L), Jackson ImmunoResearch, dilution 1:400) in 1% normal goat serum and 0.4% Triton X-100 in 1 × PBS for 30 min at room temperature. The cells were again washed in 1 × PBS for 5 min, counterstained with 4 mg/ml 4,6-diamidino-2-phenylindole, mounted in 50% glycerol, and examined with a light microscope ( Axioskop, Zeiss).

The U373MG astrocytoma cell line was obtained from Dr. H. Fine (DFCI, Boston, MA) and cultured on uncoated dishes to 60–80% confluence in DMEM (high glucose) supplemented with 10% fetal bovine serum.

The pGEM3zf'-hACT, pGEM3zf'-hAPP, and pGEM3zf'-hGAPDH constructs, respectively, contained a 407-bp PstI-SacI fragment (amino acids 175–311 in the human ACT gene (39)), a 1,056-bp EcoRI-EcoRI fragment (bp 1795–2856 in the human APP gene (40)), and a 548-bp HindIII-XmaI fragment (amino acids 66–248 in the human GAPDH gene (41)). These inserts were subcloned into the pGEM3zf' vector (Stratagene). Restriction-digested DNA from these constructs was used as a template to synthesize antisense cRNAs.

**APP Synthesis**

**Primary Astrocytes—**GFAP-positive astrocytes (1 × 10⁶ cells/well) and astrocytoma cells (70% confluent) were measured for intracellular APP synthesis and ferritin synthesis after stimulation with 0.5 ng/ml recombinant IL-1α (Genzyme), 0.5 ng/ml IL-1β (Genzyme), 10 μM ferrotransferrin (Boehringer Mannheim), 100 μM desferrioxamine (Ciba Geigy), or left untreated as controls. Cell numbers from individual wells were counted to ensure that 1 × 10⁶ cells were present in each well at the beginning of each labeling experiment. Astrocytes were preincubated for 15 min in methionine-free medium (RPMI 1640; Life Technologies, Inc.) and pulse labeled for 30 min with 300 μCi/ml [35S]methionine, after which 2 ml of medium was preincubated by centrifugation in Eppendorf tubes (10,000 rpm for 10 min), and the supernatant was immunoprecipitated with a 1:500 dilution of a COOH-terminal directed APP antibody (37, 38, 39) for 15 min, followed by 10 min washes in PBS for 5 min, followed by centrifugation in Eppendorf tubes (10,000 rpm for 10 min). The pellets were lysed using 1 ml of modified guanidinium-phenol reagent (MRC Research, Inc., Cincinnati, OH). The RNA pellets were washed twice in TES buffer (10 mM Tris, pH 7.6, 1 mM EDTA, 0.5% SDS, pH 7.6), and the A260/A280 was measured as an estimate of purity and RNA concentration. A cesium chloride procedure for the purification of RNA from astrocytes was used. Triplicate samples (10 μl) were assayed after hydrolysis of methionine charged tRNAs with 250 μl of 1 M NaOH and 1.5% H₂O₂ at 65 °C for 30 min. APP and ferritin were immunoprecipitated from U373MG astrocytoma lysates by adding 2 μl of anti-APP antibody (C-8 antibody) (42) as described for primary astrocytes.

**Immunoprecipitations—**In all labeling experiments, antigen-antibody complex was collected with protein A-Sepharose beads (Pierce) and the immunoprecipitates applied to 10–20% Tris-Tricine gels (Novex) and fractionated by electrophoresis in a buffer containing 0.1 M Tris, 0.1 M sodium chloride buffer and 0.1% SDS, pH 8.3. The gels were fixed with 25% methanol, 7% (v/v) acetic acid for 1 h, incubated with a fluorograph reagent (Amplify; Amersham Pharmacia Biotech) for 30 min, dried, and exposed to Kodak X-Omat film overnight at –80 °C.

**RNA Purification and Northern Blot Hybridization**

Equal numbers of cells were pelleted by centrifugation at 1,000 rpm and the pellets lysed using 1 ml of modified guanidinium/phenol reagent according to the manufacturer’s instructions (Tri-reagent, MRC Research, Inc., Cincinnati, OH). The RNA pellets were washed twice in TES buffer (10 mM Tris, pH 7.6, 1 mM EDTA, 0.5% SDS, pH 7.6), and the A260/A280 was measured as an estimate of purity and RNA concentration. A cesium chloride procedure for the purification of RNA from DNA was followed for all experiments involving the use of transfected DNA (36). Total RNA samples from both transfected and untransfected cells were denatured in 50% formamide, 2.2 M formaldehyde, 20 mM MOPS, 5 mM sodium acetate, 0.5 mM EDTA, pH 7.4, at 60 °C for 10 min.
FIG. 1. GFAP-positive astrocytes. Human primary astrocytes displayed immunofluorescent GFAP staining using a monoclonal antibody (clone G-A-5) and a Cy3-labeled goat anti-mouse secondary antibody (panel A) but very faint background staining following omission of primary antibody (panel B). Panels C and D, 4,6-diamidino-2-phenylindole staining of the same cells.

RNA samples were separated by electrophoresis on 1.5% agarose-formaldehyde gels, blotted onto Hybond-N filters, and immobilized by UV cross-linking (2 min) and heating of filters to 80 °C for 1 h. Filters were prehybridized overnight to reduce background hybridization signal and then hybridized overnight in 50% formamide, 50 ng/ml denatured salmon sperm DNA, 5 × SSC, 0.1% SDS, and 5 × Denhardt’s solution. After hybridization, filters were washed twice for 1 h in 2 × SSC and 0.2% SDS at room temperature. Filters were washed further through four changes of 0.5 × SSC and 0.1% SDS at 55 °C. In all experiments, equal loading of Northern gels was verified by ethidium bromide staining of 18 S and 28 S rRNAs and GAPDH hybridization as an internal standard (41). Specific RNAs were detected either by hybridization of Northern blot filters with randomly primed cDNA probes (35) or with copy RNA (cRNA) probes (37). Antisense cDNA hybridization probes were labeled by random primed labeling (specific activity 10,000 Ci/mmol, NEN Life Science Products) and subjected to three cycles of freezing and thawing (liquid nitrogen, 37 °C) to lyse the cells. Lysates were collected after centrifugation at 10,000 rpm for 5 min. The protein concentration was measured using the manufacturer’s conditions (Bio-Rad assay). Lysates (20 μg of protein) were added to 50 μl of a CAT reaction mix containing 1 μl Tris, pH 7.8, 20 μl of acetyl-CoA (3 mg/ml), and 5 μl of 14C labeled chloramphenicol (25 μCi/ml). After incubation for 1 h at 37 °C, reaction products were extracted with 1 ml of ethyl acetate, and the samples were resolved by thin layer chromatography as described previously (37). Marked areas on the TLC plate were excised and quantified by scintillation counting (Econofluor, NEN Life Science Products) using a Wallac 1409 counter (Amersham Pharmacia Biotech). The average CAT activity from a number of separate transfection experiments (see Fig. 7B) includes CAT activity as estimated by use of an assay directly counting the amount of CAT reaction product partitioning into liquid scintillation fluid (37).

Galactosidase Assay—Lysates were diluted at equal protein concentration in a CAT lysis buffer (Promega). Extracts were then incubated in the presence of ONPG color dye, and β-galactosidase enzyme activity was determined according to the manufacturer’s conditions.

RESULTS

IL-1 Stimulates APP Gene Expression at the Translational Level in Primary Human Astrocytes—APP synthesis was measured in primary human fetal brain astrocytes (95–100% GFAP-positive cells in culture (Fig. 1)) after treatment with both IL-1α and IL-1β (0.5 ng/ml) for 16 h. Fig. 2A (left panel) shows the results of a representative experiment in which 30 min of metabolic labeling with [35S]methionine was followed by APP immunoprecipitation and gel electrophoresis (n = 3). In the labeling shown, a 4-fold increase in the synthesis of intracellular APP was observed in response to a 16-h stimulus with IL-1α and a 3-fold increase in response to a 16-h stimulus with IL-1β (maximal 5.9-fold induction of APP synthesis by IL-1α for primary astrocytes (Table I)). The C-8 antibody immunoprecipitated two proteins, of 110 and 130 kDa, corresponding to the mature and immature glycosylated APP holoprotein (43). We used the rate of synthesis of other proteins as positive and negative controls to confirm that IL-1α and IL-1β specifically increase APP mRNA translation. IL-1α and IL-1β induced a 4-fold increase of H-ferritin synthesis in primary astrocytes (Fig. 2A, right panel) whereas the rate of astrocytic L-ferritin synthesis was unchanged in response to both IL-1α and IL-1β (Fig. 2A, right panel). The level of apoE protein synthesis was also determined and found to be unchanged after IL-1α or IL-1β stimulus, thus serving as an additional internal loading control to the L-ferritin for measuring specific increases in the relative rate of APP synthesis in astrocytes (n = 3) (Fig. 2B). IL-1α increased the rate of total protein synthesis by 60% (maximal increase) in primary astrocyte cultures, as measured by trichloroacetic acid precipitation of labeled proteins from triplicate lysates (Table I). The action of iron chelation with 10 μM desferrioxamine generated a similar reduction of synthesis of both L-ferritin and H-ferritin in addition to APP (Fig. 2A). Iron as ferrotransferrin had no effect on the rate of either APP...
or ferritin protein synthesis in primary astrocyte cultures. The apparent coordinate regulation of the APP and ferritin genes is discussed below in terms of the presence of homologous translational regulatory sequences in their 5'-UTRs (see Fig. 6).

Further immunoprecipitations from two additional time course experiments (2, 6, and 16 h) showed that APP synthesis increased most sharply after 6 h of stimulus of primary astrocytes with the IL-1α (Fig. 2C). Our data showed that a 6-h stimulation with IL-1α induced a maximal 5.9-fold increase in APP synthesis (Table I). A 3.8-fold increase of APP synthesis was observed after a 16-h IL-1α stimulation. β-Actin synthesis was increased by 70% (maximum) relative to unstimulated astrocytes after IL-1α stimulation (Fig. 2C). 2 h of stimulation with IL-1α increased APP synthesis by only 40% relative to untreated cells, whereas β-actin synthesis changed by 0.65-fold under the same conditions. We concluded that APP synthesis peaked at a time point between 6 and 16 h after the IL-1α stimulation of primary astrocytes. Total protein synthesis increased by 60% in response to IL-1α, as measured by trichloroacetic acid precipitation of labeled proteins from each lysate (Table I). Evidently, IL-1α increased APP synthesis by a margin 3.7-fold greater than the induction of total protein synthesis in primary astrocytes.

IL-1 also induced a 1.7-fold increase in the secretion of APP (Protease-Nexin II or APP(s)) from primary human astrocytes as measured by direct scintillation counting of labeled immunoprecipitates (Fig. 2D). Densitometry of autoradiographs from an additional experiment revealed that IL-1β induced APP(s) synthesis by 2.6-fold. In these experiments the medium was collected after a 2-h pulse labeling with [35S]methionine and
immunoprecipitated using an NH₂-terminal antibody (against amino acids 595–611 of the APP). IL-1α also enhanced secretion of APP(s) into the medium, causing a smaller 25% accumulation of APP(s). Thus, the levels of both cell-associated and secreted APP (APP(s)) were increased by exposure of primary astrocytes to IL-1.

Northern blot analysis was used to compare the possible action of IL-1α to increase the steady-state levels of APP mRNA (3 kb) in primary astrocytes over the same time course for IL-1α induction of APP synthesis (Fig. 3). We measured no increase in APP mRNA levels in primary astrocytes after 2, 6, or 16 h of IL-1α stimulation (n = 4). As a positive control for effective IL-1α signal transduction, exposure of astrocytes to the cytokine caused a pronounced increase in the steady-state levels of ACT mRNA (1.5 kb of ACT mRNA), as has been demonstrated previously (>10-fold; n = 4) (17). As Northern blot loading controls, steady-state levels of astrocytic GAPDH mRNA and 28S rRNA were unchanged after IL-1α stimulation (Fig. 3). Our data also demonstrated that not only IL-1α stimulation, but also IL-1β stimulation leaves APP mRNA levels unchanged in primary astrocytes (n = 4) (data not shown). Therefore, astrocytic APP gene expression by IL-1 is mediated by translational mechanisms.

**TABLE I**

| Hours of IL-1α stimulus | 2   | 6   | 16 |
|-------------------------|-----|-----|----|
| APP                     | 1.4 ± 0.65 | 5.9 ± 1.70 | 3.8 ± 1.70 |
| β-Actin                 | 0.5 ± 0.045 | 1.5 ± 0.11 | 1.4 ± 0.15 |
| Total protein           | 1.3 ± 0.17 | 1.6 ± 0.16 | 1.0 ± 0.22 |

**FIG. 3.** Time course showing that APP mRNA expression was unchanged in response to 0.5 ng/ml IL-1α stimulation of human primary astrocytes. Sequential Northern blot hybridizations were performed with cRNA probes against ACT mRNA (upper), APP mRNA (middle), and GAPDH mRNA (lower). The molecular weights of each RNA are: APP mRNA, 3,500; ACT mRNA, 1,500; and GAPDH mRNA, 1,000.

**FIG. 4.** IL-1α and IL-1β increase APP synthesis but have no effect on APP mRNA levels in U373MG astrocytoma cells. Panel A, cells were treated for 16 h, pulse labeled for 30 min, and derivative lysates were immunoprecipitated with COOH-terminal directed APP antibody (C-8). From left, first lane, unstimulated cells; second lane, 0.5 ng/ml IL-1α; third lane, 0.5 ng/ml IL-1β. Panel B, the same lysates immunoprecipitated with a ferritin antibody. Panel C, ACT protein synthesis after 16 h of IL-1 stimulation of astrocytoma cells. First lane, unstimulated cells; second lane, 0.5 ng/ml IL-1α; third lane, unstimulated cells; fourth lane, 0.5 ng/ml IL-1β. U373MG cells were stimulated with IL-1 for 16 h.
Similarities in APP mRNA and Ferritin mRNA Translational Control

Abbreviations

APP: amyloid precursor protein
CAT: chloramphenicol acetyltransferase
IL-1: interleukin 1
UTR: untranslated region

Fig. 5. APP synthesis in U373MG cells is induced in a dose- and time-dependent fashion by IL-1α and IL-1β stimulation in the absence of change in the steady-state levels of APP mRNA. Panel A, dose-response experiment measuring APP synthesis. Cells were treated and lysates harvested and immunoprecipitated with COOH-terminal directed APP antibody (C-8) as described in Fig. 4. Left group: from left, first lane, unstimulated cells; second lane, 0.05 ng/ml IL-1α; third lane, 0.5 ng/ml IL-1α; fourth lane, 5 ng/ml IL-1α. Right group: from left, first lane, unstimulated cells; second lane, 0.05 ng/ml IL-1β; third lane, 0.5 ng/ml IL-1β; fourth lane, 5 ng/ml IL-1β. Panel B, dose-response experiment measuring the steady-state levels of APP mRNA. Northern blot hybridization was performed with cDNA probe complementary to APP mRNA sequences. Lane 1, unstimulated cells; lane 2, 0.05 ng/ml IL-1α; lane 3, 5 ng/ml IL-1α; lane 4, 0.05 ng/ml IL-1β; lane 5, 5 ng/ml IL-1β. Panel C, left group, time course experiment measuring APP synthesis. Cells were treated, and lysates were harvested and immunoprecipitated with COOH-terminal directed APP antibody (C-8) as described in Fig. 4A. From left, first lane, unstimulated cells; second lane, 0.5 ng/ml IL-1β stimulation for 2 h; third lane, 0.5 ng/ml IL-1β stimulation for 6 h. Right group, time course experiment measuring the steady-state levels of APP mRNA. Northern blot hybridization was performed using a labeled cDNA probe against APP mRNA. First lane, in vitro translated APP mRNA marker (0.5 kb); second lane, unstimulated cells; third and fourth lanes, 0.5 ng/ml IL-1β, 2 and 6 h, respectively.

precipitations of astrocytoma cells stimulated with 5 ng/ml IL-1α for 16 h (Fig. 5A). Two dose-response experiments showed that all three concentrations of IL-1α and IL-1β generated an average 12-fold increase of APP synthesis in U373MG cells (Table II, n = 2). In the same experiments, IL-1 elevated total protein synthesis by only 2–3-fold in U373MG cells, as measured by [35S]methionine incorporation into trichloroacetic acid-insoluble counts (Table II). To confirm translational regulation, IL-1 stimulation did not increase APP mRNA levels over the same 0.05–5 ng/ml concentration range of IL-1α and IL-1β used to generate a 12-fold increase APP synthesis (Fig. 5B).

Additional time course experiments (2 h and 16 h) with astrocytoma cells reflected closely the pattern of translational regulation of APP gene expression by IL-1 observed in primary astrocytes (as shown in Fig. 2C). In U373MG cells, IL-1β (0.5 ng/ml) increased APP synthesis starting 6 h after cytokine stimulation (Fig. 5C, left panel). APP levels were unchanged after 2 h stimulation. By contrast, the steady-state levels of APP mRNA were unchanged at all time points after IL-1β stimulation (Fig. 5C, right panel). Multiple immunoprecipitation experiments demonstrated that IL-1α and IL-1β each generated an overall average 5-fold and 9-fold induction of APP synthesis in astrocytoma cells (n = 7). These data confirm that 1) IL-1 regulates APP synthesis at the level of message translation in astrocytoma cells; that 2) IL-1-dependent translational regulation of astrocytoma APP mRNA begins after 6 h of cytokine stimulation, reflecting the induction profile of APP mRNA translation in primary astrocytes; and that 3) IL-1 induction of APP synthesis is 2–5-fold greater than the induction of total protein synthesis in astrocytoma cells.

An IL-1-dependent Translational Enhancer in the APP mRNA 5′-UTR—A consistent feature of our cell culture labeling experiments was that IL-1 and iron chelation with desferrioxamine generated a similar profile for APP and ferritin synthesis (Fig. 2). Previously, the 5′-UTRs of the L-ferritin and H-ferritin genes (+74 bp to +142 bp from the L-ferritin gene cap site and +139 bp to +199 bp from the H-ferritin gene cap site) have been shown to confer both baseline and IL-1-dependent translation to a CAT reporter gene transfected in human hepatoma cells (37). Therefore we aligned L- and H-ferritin gene 5′-UTR sequences with the APP gene. Fig. 6 shows the presence of an unexpectedly high 51% sequence homology sequence alignment between the L-ferritin and APP mRNA 5′-UTRs (Gap program, Genetics Software from University of Wisconsin, Madison). Because the APP mRNA 5′-UTR contained a significant sequence homology to IL-1-responsive 5′-UTR translational regulatory sequences in both L- and H-ferritin mRNAs (+85 bp to +146 bp from the 5′-cap site of the APP gene; Fig. 6), we tested whether these APP mRNA 5′-UTR sequences could confer IL-1-dependent translational enhancement. A 90-nucleotide DNA fragment, encoding sequences from positions +55 to +144 between the SmaI to NruI sites of the APP gene 5′-UTR, was inserted immediately upstream of a hybrid CAT reporter gene. The resulting reporter construct was designated as pSV2(APP)CAT because it was a derivative of the pSV2-CAT expression vector.

Multiple transfection experiments with the pSV2(APP)CAT construct showed that both IL-1α and IL-1β, respectively, conferred an average 3-fold and 4-fold translational enhancement to CAT reporter mRNAs in U373MG astrocytoma cells (n = 6) (Fig. 7B). Panel A shows a duplicate experiment where IL-1α increased CAT gene expression by 6-fold, and IL-1β increased CAT gene expression by 9-fold in pSV2(APP)CAT-transfected astrocytoma cells. This induction was sufficient to account for a significant proportion of the IL-1-enhanced APP synthesis in astrocytoma cells. As a negative control, IL-1β stimulation of pSV2(CAT)-transfected astrocytoma cells did not increase CAT activity, confirming that the APP mRNA 5′-UTR is a translational regulatory element (36). In the representative experiment shown in Fig. 7C no sequences in the parental vector pSV2(CAT) conferred IL-1-dependent translational regulation. At the same time CAT activity was enhanced 3-fold in pSV2(APP)CAT-transfected Northern blot analysis confirmed that a 16-h exposure to both IL-1α and IL-1β (0.5 ng/ml) did not significantly change the steady-state levels of the transfected APP/CAT hybrid mRNA in pSV2(APP)CAT-transfected astrocytoma cells (Fig. 7D). Purified RNA from either pSV2(APP)CAT or pSV2(CAT) (negative control) transfecteds was hybridized to labeled antisense RNA sequences homolo-
gous to the 5'-end of the CAT gene. The pSV2CAT transfectants expressed a 1,527-nucleotide CAT mRNA as expected (Fig. 7D, lanes 1 and 2, shows two separate loadings, 10 and 2 μg, respectively). The pSV2(APP)CAT-transfected cells expressed a closely migrating APP/CAT mRNA (1,617 nucleotides), larger by the presence of the 90-nucleotide insert coding for the APP gene 5'-UTR, but also transcribed another larger (1,640 nucleotides) APP/CAT transcript (Fig. 7D, lane 3). This APP/CAT mRNA was likely the result of using a second poly(A) addition site downstream from the CAT gene stop codon in pSV2CAT.

IL-1α and IL-1β stimulated the reappearance of only the single 1,617-nucleotide APP/CAT mRNA transcript using the upstream poly(A) addition site. Densitometric quantitation of autoradiographs showed that IL-1α or IL-1β only modestly (30%) increased the total quantity of APP/CAT mRNA transcribed in pSV2(APP)CAT transfectants relative to standardizing GAPDH mRNA levels (Fig. 7D, lanes 3–5). Slot-blot analysis has convincingly confirmed that neither IL-1α nor IL-1β altered the steady-state levels of APP/CAT mRNA in pSV2(APP)CAT-transfected astrocytoma (U373MG) cells (data not shown).

**TABLE II**

| IL-1α concentration (ng/ml) | IL-1β concentration (ng/ml) |
|-----------------------------|-----------------------------|
| 0.05 | 0.5 | 5.0 | 0.05 | 0.5 | 5.0 |
| Total protein (n = 3) | 2.1 ± 0.24 | 2.4 ± 0.30 | 2.0 ± 0.30 | 2.9 ± 0.03 | 3.1 ± 0.22 | 2.2 ± 0.15 |
| APP (n = 2) | 7.15 | 8.7 | 12 | 7.8 | 11 | 12.3 |

**FIG. 6.** Hybrid CAT constructs expressing the APP mRNA 5'-UTR. Upper panel, the pSV2(APP)CAT construct was prepared by inserting a 90-bp Smal-NruI fragment of the APP gene 5'-UTR immediately upstream of the CAT mRNA start codon. Computer alignment between the 5'-UTR of the APP gene and the IL-1-responsive 5'-UTR translational enhancer in L-ferritin mRNA revealed 51% sequence homology (bold lettering). The acute box homology motif is underlined (36). Lower panel, comparison of the predicted folding of RNA by computer analysis of the APP mRNA 5'-UTR and the IL-1-responsive L-ferritin mRNA 5'-UTR translational enhancer (37). The APP mRNA acute box sequence is predicted to fold into a stable RNA stem-loop structure (47). This RNA stem-loop is identical to a larger stem-loop folded from the complete APP mRNA 5'-UTR (∆G = −54 kCal/mol). The corresponding L-ferritin mRNA 5'-UTR stem-loop, specific to the acute box consensus, folds into a less stable RNA structure (∆G = −16 kCal/mol).
not stimulated; third lane, unstimulated cells; fourth lane, 0.5 ng/ml IL-1α; fifth lane, 0.5 ng/ml IL-1β. The lysates were normalized for transfection efficiency using 5 μg of a reference RSV_GAL plasmid. Panel D, Northern blot hybridizations of RNA purified from pSV2CAT and pSV2(APP)CAT-transfected astrocytoma cells (control and IL-1-stimulated) with a labeled cRNA probe complementary to the 5′-end coding sequences and 5′-UTR of the CAT gene from the pSBGAT subclone (36). Lanes 1 and 2, 10 and 2 μg of RNA from astrocytoma cells transfected with the parental vector (pSV2CAT); lanes 3–5, 10 μg of RNA from astrocytoma cells transfected with the pSV2(APP)CAT vector and treated for 16 h as follows: lane 3, unstimulated cells; lane 4, 0.5 ng/ml IL-1α; lane 5, 0.5 ng/ml IL-1β. The Northern blot shown was standardized for loading by use of a GAPDH gene probe. The ratio of IL-1 induction of APP/CAT to GAPDH mRNA established that IL-1 only increased the overall expression of transfected APP/CAT mRNA by a 30% margin.

**DISCUSSION**

This report provides the first evidence that IL-1 substantially induces APP synthesis in primary human astrocytes and astrocytoma cells by a mechanism of enhanced message translation. The translational efficiency of astrocytic APP mRNA was specifically and selectively enhanced by IL-1, while the translational efficiencies of the astrocytic mRNAs for β-actin, L-ferritin, and apoE were unaffected (H-ferritin synthesis is increased in astrocytes (Figs. 2A and 4B)). Induced APP synthesis was not observable after 2 h, but required 6 h of IL-1β stimulation in both primary astrocytes and in U373MG astrocytoma cells. IL-1 was shown to increase total astrocytoma protein synthesis by 2–3-fold, similar to insulin signaling of protein synthesis in HEK293 cells (44). The cytokine specifically induced a more substantial level of APP synthesis (Tables I and II). A similar time course of increased L- and H-ferritin mRNA translation during inflammation has been demonstrated in rat liver cells (45). The expression ratio of APP isoforms (APP-695:APP-751:APP-770) in astrocytes is 1:4:2 (i.e., APP-695 predominates in neuronal cells (2, 42, 43).)

Both cytokine isoforms, IL-1α and IL-1β, increased APP synthesis, although IL-1β enhanced the secretion of APP(s) to a greater extent than IL-1α. Differences in the magnitude of...
cytokine-stimulated secretion of [35S]methionine-labeled APP(s) and nascent APP are likely the result of additional actions by IL-1 to alter the APP processing (1, 46). It has been shown previously that the effect of IL-1β on APP processing in human umbilical vein endothelial cells is mediated by the IL-1 receptor (46). The two cytokines are known to differ in a number of biological responses that they illicit (28). IL-1α is a cell-associated cytokine expressed as a fully active 31-kDa precursor protein (pro-L-1α) that is cleaved into a mature 17-Da IL-1α product. In contrast, IL-1β operates at the systemic level, where only the cleaved 17-Da cytokine is active. Additionally, only IL-1β preferentially binds to the IL-1 receptor II, perhaps also modifying signal transduction though the IL-1 receptor I (28). IL-1α does not bind at a high affinity to IL-1 receptor II.

We identified a novel IL-1-responsive and basal translational enhancer in the 5’-UTR of the APP gene, consistent with computer alignment with similar 5’-UTR sequences in the ferritin genes. Ferritin gene expression has been well characterized and is known to be regulated at the level of message translation in hepatoma cells (37). Transfection studies with a hybrid APP/CAT mRNA construct confirmed that the action of this sequence, mapping from +55 to +144 nucleotides from the APP mRNA 5’-cap site, was sufficient to mediate the translational regulation of APP mRNA by IL-1 in U373MG cells, as measured by CAT reporter activity. In contrast, the steady-state levels of transfected hybrid APP/CAT mRNA was unchanged, similar to findings from parallel CAT reporter studies with the ferritin mRNA acute box elements (36). The most straightforward interpretation of our results is that IL-1 elevates APP mRNA translation through the action of an IL-1-responsive stem-loop structure in APP mRNAs. Computer alignment showed that sequences in the 5’-UTR of APP mRNAs are homologous, but not identical, to the acute box sequence of L-ferritin mRNA 5’-UTR which confers IL-1-dependent translation specifically in hepatoma cells. The L-ferritin mRNA sequence differs from the APP mRNA sequence, likely explaining the lack of L-ferritin gene translational regulation by IL-1 in astrocytoma cells. The APP mRNA 5’-UTR sequence is highly GC-rich (80%) and is predicted to fold into a single stable RNA stem-loop structure ($\Delta G = -54$ kCal/mol in

FIG. 8. Top histogram, representative transfection experiment showing that the APP gene 5’-UTR sequences conferred a 3.8-fold increased basal CAT gene expression in U373MG cells transfected with pSV2(APP)CAT compared with the parental pSV2CAT vector (ratio of 1.7 to 6.4% acetylation ($n = 2$). Lower histogram, quantitation of multiple transfection experiments showing that the APP gene 5’-UTR sequences conferred 4.3-fold increased basal CAT gene expression in SKN-SH neuroblastoma cells transfected with pSV2(APP)CAT compared with the parental pSV2CAT vector ($n = 6$). Differences in transfection efficiency were normalized using a reference RSV2GAL plasmid (mean ± S.E. $n = 6$).
There are striking overlaps in the regulation of the APP gene and the L- and H-ferritin genes, each of which encodes the subunits for the central iron storage protein shown in Fig. 2. APP mRNA 5′-UTR sequences confer significant IL-1-dependent and basal translational enhancement to activate CAT reporter gene expression in pSVβAPP/CAT-transfected astrocytoma cells. Similar hepatic translational regulation is conferred by the IL-1-responsive acute box RNA sequences in the L and H-ferritin mRNA 5′-UTRs (37). Like the ferritin genes, the APP 5′-UTR maintains efficient translation of APP in both astrocyte-derived and neuroblastoma cells. The L- and H-ferritin gene 5′-UTRs are organized into two regulatory sequences: an iron-responsive element at the 5′-cap site, which is responsive to iron (48), oxidative stress (49), phorbol esters (50) and thyroid hormone receptor (51); and a downstream acute box sequence that is both a base-line and an IL-1-dependent translational regulatory element that works in an iron-dependent fashion (Fig. 4B) (35). The presence of similar translational regulatory sequences in the 5′-UTRs of both APP mRNA and ferritin mRNA is consistent with the known role for metal binding, including copper and likely iron, as a part of the normal function of APP in cells (52). APP mRNA 3′-UTR sequences regulate APP gene expression by modulating message stability in human peripheral blood mononuclear cells (53) and regulating message translation in Chinese hamster ovary cells (54). In addition, other studies have indirectly shown translational regulation of APP gene expression. The steady-state levels of APP protein in the rat cerebral cortex, meninges, and in primary astroglial, microglial, and neuronal cultures have been reported not to reflect APP mRNA levels (55). Furthermore, the relative levels of APP-695 (KPI−) and APP-751 (KPI+) mRNA and their proteins have been found to be discordant in human brain. Each transcript was approximately equally abundant, whereas KPI+ proteins predominated (>92%) and at elevated levels in the Alzheimer’s brain (56, 57).

Several reports suggest a direct connection between increased APP levels and the development of AD pathogenesis. This increase might be linked to inflammatory mechanisms. 1) Down’s syndrome brains and trisomy 16 mice show increased APP levels and the development of AD pathogenesis. This increase might be linked to inflammatory mechanisms. 1) Down’s syndrome brains and trisomy 16 mice show increased APP levels and the development of AD pathogenesis. This increase might be linked to inflammatory mechanisms. 2) Increased APP synthesis by enhanced message translation increases acute box RNA sequences in the L- and H-ferritin mRNA (48). 2) Increased APP synthesis by enhanced message translation increases acute box RNA sequences in the L- and H-ferritin mRNA (48). 3) Iron, oxidative stress (49), phorbol esters (50) and thyroid hormone receptor (51); and a downstream acute box sequence that is both a base-line and an IL-1-dependent translational regulatory element that works in an iron-dependent fashion (Fig. 4B) (35). The presence of similar translational regulatory sequences in the 5′-UTRs of both APP mRNA and ferritin mRNA is consistent with the known role for metal binding, including copper and likely iron, as a part of the normal function of APP in cells (52). APP mRNA 3′-UTR sequences regulate APP gene expression by modulating message stability in human peripheral blood mononuclear cells (53) and regulating message translation in Chinese hamster ovary cells (54). In addition, other studies have indirectly shown translational regulation of APP gene expression. The steady-state levels of APP protein in the rat cerebral cortex, meninges, and in primary astroglial, microglial, and neuronal cultures have been reported not to reflect APP mRNA levels (55). Furthermore, the relative levels of APP-695 (KPI−) and APP-751 (KPI+) mRNA and their proteins have been found to be discordant in human brain. Each transcript was approximately equally abundant, whereas KPI+ proteins predominated (>92%) and at elevated levels in the Alzheimer’s brain (56, 57).

Several reports suggest a direct connection between increased APP levels and the development of AD pathogenesis. This increase might be linked to inflammatory mechanisms. 1) Down’s syndrome brains and trisomy 16 mice show increased APP levels beyond the 0.5-fold increase that would be expected in vivo (59, 60). Furthermore, APP synthesis correlates with Aβ secretion in vitro (61). 2) Traumatic brain injury, a known risk factor for AD, increases IL-1 as well as APP immunoreactivity in rat brain (20, 32). 4) IL-1 injected into the rat cerebral cortex increases the steady-state levels of APP protein at the site of the lesion (62), and primary astrocytes have been shown to be a source of secreted Aβ peptides (63).

Overexpression of IL-1 by centrally located microglia has been shown to be associated even with early forms of amyloid plaques, the non-neuritic diffuse plaques, as well as being increased strikingly during plaque development (17, 18, 62). IL-1 has been suggested as a driving force for amyloid plaque maturation (62), perhaps mediated by signaling by the cytokine to astrocytes surrounding the plaque structures and subse-
