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Interleukin 1 regulates synthesis of amyloid β-protein precursor mRNA in human endothelial cells

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ABSTRACT **We have analyzed the modulation of amyloid β-protein precursor (APP) gene expression in human umbilical vein endothelial cells (HUVEC). The level of the APP mRNA transcripts increased as HUVEC reached confluency. In confluent culture the half-life of the APP mRNA was 4 hr. Treatment of the cells with human recombinant interleukin 1 (IL-1), phorbol 12-myristate 13-acetate, or heparin-binding growth factor 1 enhanced the expression of *APP* gene in these cells, but calcium ionophore A23187 and dexamethasone did not. The protein kinase C inhibitor 1-(isoquinolinsulfonyl)-2-methylpiperazine (H7) inhibited IL-1-mediated increase of the level of APP transcripts. To map IL-1-responsive elements of the *APP* promoter, truncated portions of the *APP* promoter were fused to the human growth hormone reporter gene. The recombinant plasmids were transfected into mouse neuroblastoma cells, and the cell medium was assayed for the human growth hormone. A 180-base-pair region of the *APP* promoter located between position −485 and −305 upstream from the transcription start site was necessary for IL-1-mediated induction of the reporter gene. This region contains the upstream transcription factor AP-1 binding site. These results suggest that IL-1 upregulates *APP* gene expression in HUVEC through a pathway mediated by protein kinase C, utilizing the upstream AP-1 binding site of the APP promoter.**

The amyloid β-protein precursor (APP) gene codes for a family of proteins, each containing a 42-amino acid fragment called amyloid β or A4 protein, that is found in brains and the cerebrovasculature of Alzheimer disease, Down syndrome, hereditary cerebral hemorrhage (Dutch type), and aging (1–5). The *APP* gene is transcriptionally active in a variety of tissues, highly conserved in evolution, maps to human chromosome 21 (6–10), and probably has no mutations associated with Alzheimer disease (11, 12). Transcripts containing three similar but nonidentical open reading frames have been identified (13–17). *In situ* hybridization studies show a very complex pattern of the *APP* gene expression in brain (18–23). Different levels of APP mRNAs were detected in neurons, some glial cells, and cells that compose blood vessels, presumably some endothelial cells (23). The detection of APP mRNA in these cells strengthens the possibility that at least one form of amyloid β-protein—cerebrovascular—could originate locally from blood vessels (24, 25).

The importance of understanding the regulation of the *APP* gene is underscored by two observations. First, there are differences between Alzheimer disease patients and controls in the levels of APP mRNAs in certain brain regions (19, 23, 26–30). Second, the difference in the level of APP mRNAs between trisomy 21 Down-syndrome patients and controls having two chromosomes 21 was higher than the expected 3:2 ratio (9). In the absence of structural mutations, aberrant levels of the precursor protein may contribute to pathology. These observations suggest that there is aberrant regulation of the *APP* gene in Alzheimer disease and Down syndrome patients. Further, the striking similarity of neuropathological changes in Alzheimer disease and Down syndrome implies a common multistep mechanism of amyloid formation that may involve changes in the *APP* gene expression as a required step.

The goal of this study was to identify factors that regulate the level of APP mRNAs in human endothelial cells. We describe here the expression of APP mRNA transcripts by human endothelial cells, the responsiveness of the *APP* gene to interleukin 1 (IL-1) and several other mediators, and a localization of the cis-acting regulatory element that plays a key role in the induction of the *APP* gene expression by IL-1.

MATERIALS AND METHODS

Cell Cultures and DNA Transfection. Human umbilical vein endothelial cells (HUVEC) were cultured as described (31) in 75-cm^2 tissue culture flasks coated with fibronectin (10 μg/cm) in medium 199 (GIBCO) supplemented with 10% (vol/vol) fetal calf serum, 100 μg of a crude preparation of heparin-binding growth factor-1 (HBGF-1) (32) per ml, and 5 units of heparin (Sigma) per ml. HUVEC were used between passage 5 and 10 for all experiments. Except where noted, upon reaching confluence, the cells were maintained for 24 hr in 5% fetal calf serum without HBGF-1 or heparin. AB-1 is a mouse neuroblastoma cell line obtained from M. Notter (University of Rochester). AB-1 cells were plated (10^6 per 35-mm dish) in Ham's F-12 medium supplemented with 10% fetal calf serum. Four 35-mm dishes with semiconfluent AB-1 cells were used in each experiment. Ten micrograms of plasmid DNA was precipitated in calcium phosphate (33) and left on the cells for 4 hr. After the cells were washed and the medium was changed, IL-1β or IL-1α at 1 ng/ml was added to one pair of each quadruplicate group, and 56 hr later two 100-μl aliquots of medium from each culture were tested by a radioimmunoassay for human growth hormone (hGH) with a kit from Nichol's Diagnostics (San Juan Capistrano, CA).

Mediators. Recombinant human HBGF-1 was a gift of R. Forough (American Red Cross), and the details on the recombinant polypeptide will be reported elsewhere. Recombinant human IL-1α was obtained from P. Lomedico (Hoffmann–La Roche). IL-1β was obtained from ICN. Calcium ionophore A23187, dexamethasone, actinomycin D, phorbol 12-myristate 13-acetate (PMA), and 1-(isoquinolinsulfonyl)-2-methylpiperazine (H7) were obtained from Sigma.

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Abbreviations: APP, amyloid β-protein precursor; HUVEC, human umbilical vein endothelial cells; IL-1, interleukin 1; PMA, phorbol 12-myristate 13-acetate; HBGF-1, heparin-binding growth factor 1.

RNA (Northern) Blot Analysis. Total cellular RNA was isolated as described (31). Briefly, cells (2×10^7) were washed with phosphate-buffered saline and lysed in 2 ml of 4 M guanidinium isothiocyanate/0.5% *N*-lauroylsarcosine/0.2 M Tris, pH 8.0/0.1 M 2-mercaptoethanol. The lysate was extracted twice with phenol/isoamyl alcohol/chloroform, 24:1:24 (vol/vol), and nucleic acids were precipitated with equal volumes of isopropyl alcohol. After DNase digestion, 10-μg samples of RNA were denatured in 2.2 M formaldehyde/50% (vol/vol) formamide and subjected to electrophoresis in a 1.25% agarose gel containing 2.2 M formaldehyde. These gels were stained with ethidium bromide at 0.5 μg/ml and photographed to verify that each lane contained an approximately equal amount of undegraded RNA. After transfer to Zeta-Probe nylon membranes (Bio-Rad), the membranes were hybridized to ^{32}P-labeled APP-specific probes. One probe, a 1.05-kilobase (kb) *Eco*RI fragment of a phage λ Am4 APP cDNA clone (6), detected a common region in APP mRNAs. Probes were labeled by using a randomly primed DNA labeling kit from Boehringer Mannheim (34). After hybridization the membranes were washed under conditions of high stringency as described (35) except that prehybridization solutions contained 2% sodium dodecyl sulfate (SDS). After exposure of hybridization membranes to XAR-5 film (Kodak), they were stripped by incubation in 0.1% SDS/20% formamide at 80°C for 15 min. Stripped filters were rehybridized to a 1.0-kb human β-actin or an 18S ribosomal RNA probe to verify quantitation of RNA. The β-actin probe was a gift from Stephen Chang (National Institute of Neurological and Communicative Disorders and Stroke). Autoradiograms were scanned with a dual-wavelength densitometer (Shimadzu, Kyoto, Japan).

Plasmid Constructions. Phage λ clone 783 containing the human *APP* gene promoter was isolated from a chromosome 21 genomic library (ATCC 57743; R.J.D. and M.P.V., unpublished data). The *APP* promoter was first cloned into the *Eco*RI site of pUC19, followed by subcloning into the *Bam*HI site of pOGH plasmid, which lacks a promoter but contains five exons of the gene for hGH (36). One construct, pCLL-EB, spans position numbers −2830 to +101 [adenosine in the first AUG of APP_{695} cDNA (7) is at position +147], while the second, pCLL-BE, contains the same fragment in the inverse (+101 to −2837) orientation. Two additional clones were derived from the pCLL-EB construct by restriction digestion with *Hin*dIII or with *Xba* I endonucleases followed by religation of the shortened plasmids and were named pCLL-HB (−485 to +101) and pCLLXB (−305 to +101), respectively. All positions are numbered according to Salbaum *et al.* (37).

RESULTS

To evaluate the effect of growth on *APP* gene expression, the levels of APP mRNA transcripts were determined in HUVEC cultures grown at various densities. The expression of the APP mRNA transcripts was minimal during phases of active growth (low cell density) and increased in confluent cultures (Fig. 1*A*). Cells harvested 1 day after subculture at a 1:2 or 1:4 split ratio demonstrated the equally low level of the APP mRNA transcripts. Thus, the density at which the cells were grown does not appear to influence APP expression.

To study the turnover rate of APP mRNA transcripts, confluent cultures of HUVEC were treated with actinomycin D, and RNA was isolated from the cells harvested at various time points, electrophoresed, and probed with the *APP* probe. The quantity of APP mRNA transcripts decreased logarithmically with a half-life of ≈4 hr (Fig. 1*B*). Changes in the level of the APP mRNA transcripts after treatment with several mediators known to modulate gene expression in vascular endothelial cells are shown in Fig. 2*A*. PMA, IL-1,

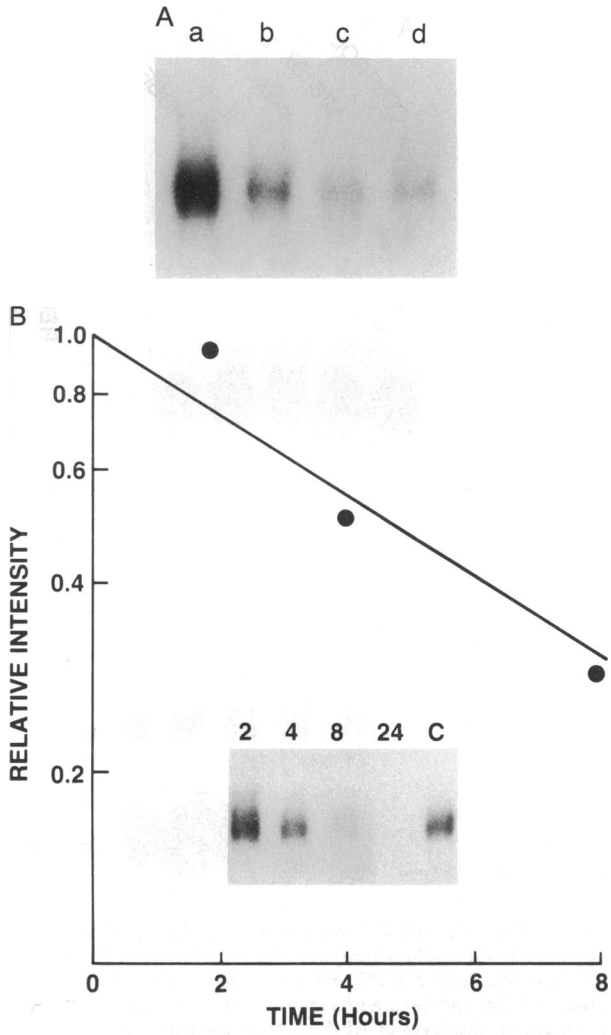

FIG. 1. RNA blots of APP mRNAs from human endothelial cells. Total RNA was extracted from HUVEC, subjected to electrophoresis on a formaldehyde/agarose gel, and hybridized with labeled Am4 probe. (*A*) Effect of confluency on the level of APP mRNAs. Lanes: a, RNA from a confluent culture 96 hr after plating; b, RNA from a confluent culture 72 hr after plating; c, RNA from a culture 24 hr after plating at a 1:4 ratio; d, RNA from a culture 24 hr after plating at a 1:2 ratio. (*B*) Effect of actinomycin D on the level of APP mRNAs. HUVEC were incubated with 10 μg of actinomycin D per ml, and cells were harvested 2, 4, 8, or 24 hr later. A semilogarithmic plot of relative change in APP mRNAs as a function of time is based on densitometric scanning. The Northern blot is shown below.

and HBGF-1 induced a 3- to 4-fold increase in the level of APP mRNA transcripts in confluent monolayers of HUVEC. The calcium ionophore A23187 and dexamethasone did not change the level of the APP mRNA transcripts (Fig. 2*B*).

The active physiologic mediators IL-1 and HBGF-1 were selected for kinetic analysis. Normalized results from these experiments are shown in Fig. 2*C*. IL-1 had a relatively rapid onset of action, reaching peak levels between 6 and 24 hr. The effect of IL-1 then began to level off. In contrast, HBGF-1 had a delayed onset and more prolonged course of action.

The increase in the level of the APP mRNA by PMA suggests that activation of the protein kinase C pathway (38) may play an important role in the regulation of *APP*. It is known that in some cell systems, IL-1 acts through the protein kinase C pathway (39). It seemed likely that the effects of IL-1 on the levels of the APP mRNA transcripts in HUVEC were also mediated by protein kinase C. Therefore, the protein kinase C inhibitor H7 (40) was used in combina-

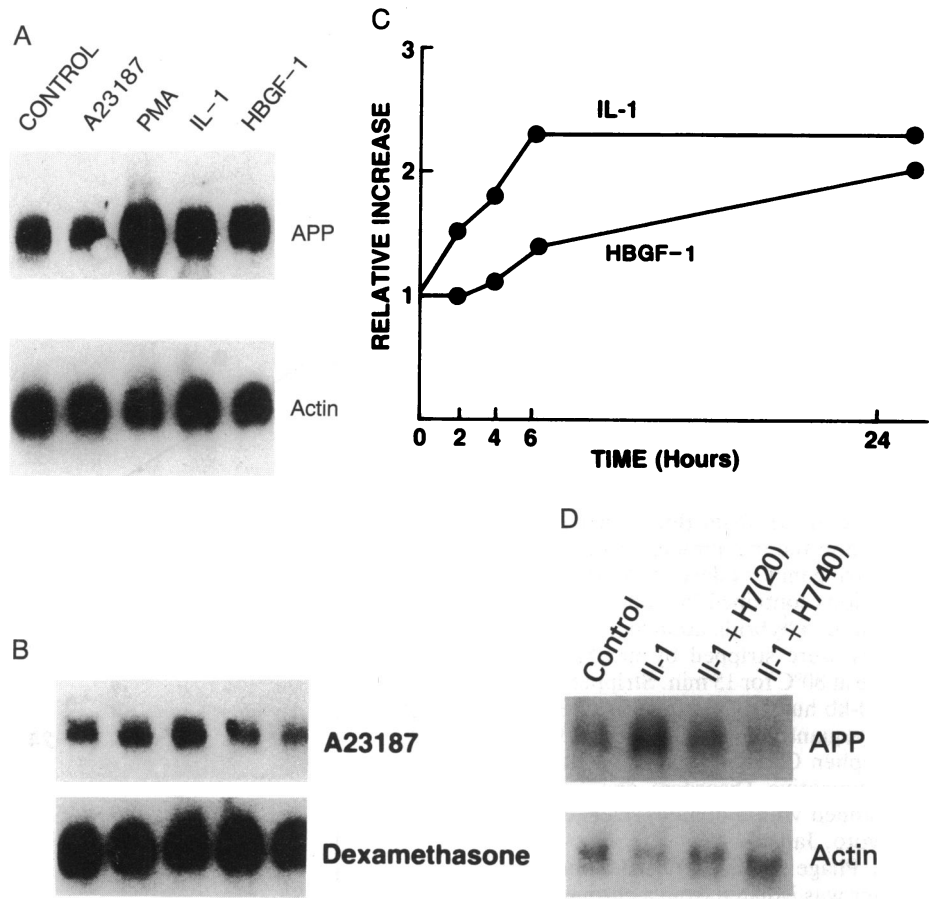

FIG. 2. RNA blots of APP mRNAs from human endothelial cells. (*A*) Total RNA was extracted from cells incubated for 24 hr with no addition (Control), 100 nM calcium ionophore A23187, 300 ng of PMA per ml, 30 ng of IL-1 per ml, or 20 ng of HBGF-1 per ml. (*B*) Dose–response effects of calcium ionophore A23187 and dexamethasone on the level of APP mRNAs. Lanes from left to right: A23187 at 0 nM, 0.1 nM, 1.0 nM, 10 nM, and 100 nM; and dexamethasone at 0 nM, 0.02 nM, 0.2 nM, 2.0 nM, and 20.0 nM. (*C*) Time course of effects of IL-1 at 200 ng/ml and HBGF-1 at 20 ng/ml on the level of APP mRNAs. Densitometric analysis of Northern blots of APP mRNAs were normalized to the actin signal. (*D*) Inhibitory effect of H7 on the IL-1-induced increase in the level of APP mRNAs. HUVEC were treated with IL-1 (200 ng/ml) alone or in the presence of 20 μM H7 or 40 μM H7.

tion with IL-1 to determine whether this pathway is involved in *APP* gene regulation. The H7 inhibitor did eliminate the IL-1-induced increase in the level of the APP mRNAs in a dose-dependent manner (Fig. 2*D*).

The promoter region of the *APP* gene has recently been identified (37). The *APP* promoter region does not contain a TATA box, has a high G+C content and a heat shock control element, and contains two regions with homology to the consensus sequence recognized by the transcription factor AP-1. However, the functionality of these AP-1 sites is not known. To map the *APP* promoter-active region that is responsive to the induction by IL-1, a number of the *APP* promoter fragments were fused with the hGH cDNA (Fig. 3*A*) that was subcloned into the pOGH plasmid (36), and each of the constructs was separately transfected into AB-1 mouse neuroblastoma cells. The cells were grown with or without IL-1. The quantity of the released hGH was measured in the medium of the transfected AB-1 cells. The results show that IL-1 can induce hGH gene expression that is driven by the *APP* promoter (Fig. 3*B*). It also shows that the removal of the 180-bp fragment containing the upstream AP-1 binding site (position −350) eliminated the induction of the reporter hGH gene by IL-1.

DISCUSSION

The experiments described above suggest that the level of expression of the APP mRNA transcripts in human endothelial cells can be modulated by PMA, IL-1, and HBGF-1 but not by calcium ionophore A23187 or dexamethasone. IL-1 is a major modulator of the immune response to trauma, infection, or inflammation (41). Endothelial cells exposed to IL-1 synthesize prostaglandins and platelet-activating factor (40) and show accelerated release of von Willebrand factor (42). Interestingly, a significant increase in formation of prostaglandin D_2 in frontal cortex of Alzheimer disease patients was described recently (43). Systemic administration of IL-1 results in increased hepatic production of serum amyloid A, a precursor of the amyloid fibrils found in a secondary amyloidosis (44). IL-1 is expressed in the central nervous system (45) where it is thought to play a number of roles, including a hypothalamic acute-phase response (46) and a stimulation of astroglial proliferation after brain injury (47). Because of the diverse biological activities of IL-1, the observation that IL-1 enhances expression of the APP mRNA transcripts in human endothelial cells is of particular importance. Griffin *et al.* (48) described the increased level of IL-1 in brains of patients with Alzheimer disease and Down syndrome. Thus, the conditions that may lead to the increased expression of the *APP* gene are present in both diseases.

IL-1 induced a relatively rapid increase in the level of the APP mRNA transcripts. Because the half-life of the transcripts is short, these changes may either reflect transcriptional rate changes or modification in the posttranscriptional half-life of the transcript. The increase in the level of APP

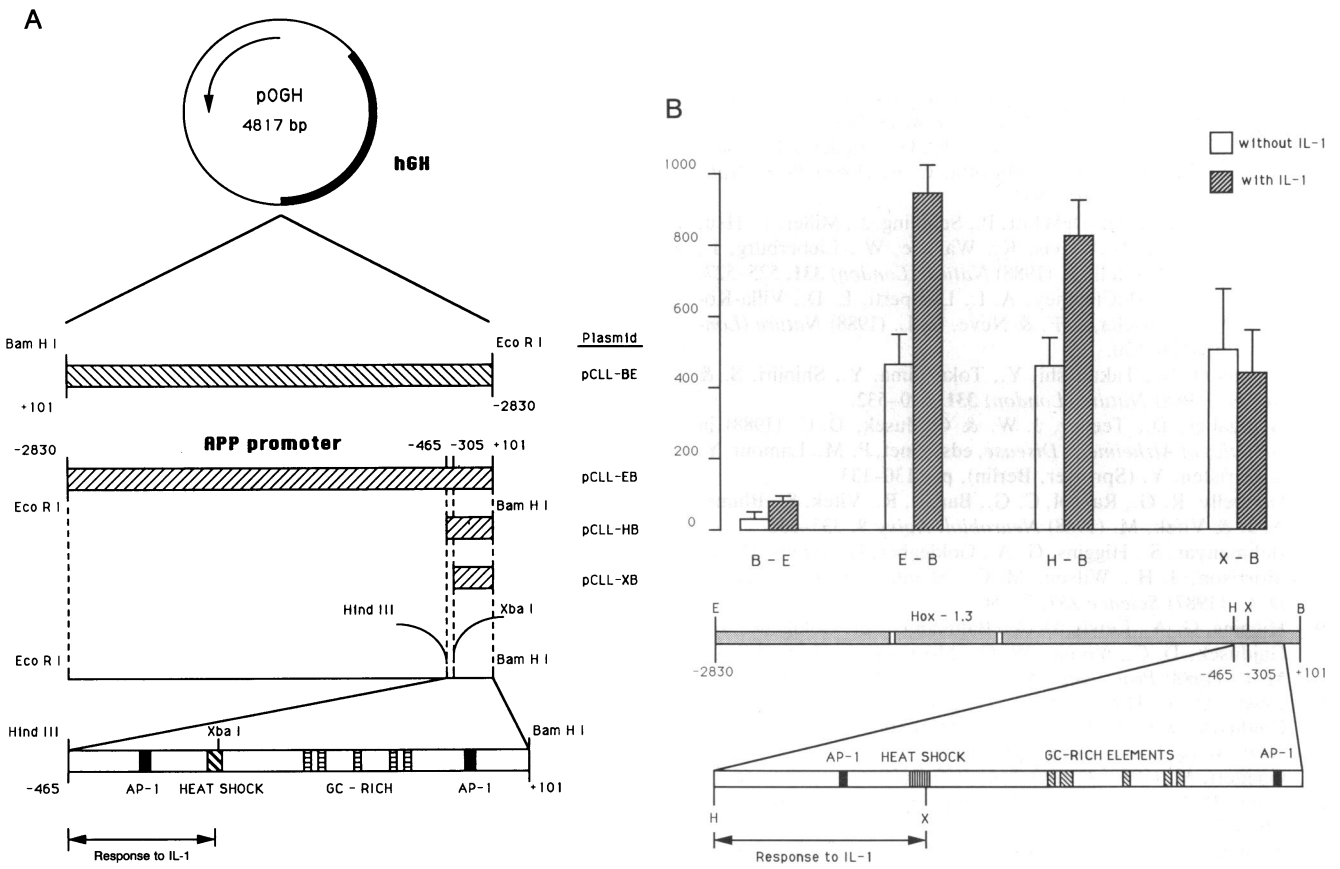

FIG. 3. Schematic representation of pOGH vectors containing *APP* promoter fragments fused to a reporter hGH gene (*A*) and quantitation of human growth hormone (hGH) in the medium of AB-1 cells transfected with different *APP* promoter/hGH constructs (*B*). (*A*) In the diagram a promoterless hGH gene is shown in black. The hatched area corresponds to the *APP* promoter fragments. Numbers indicate positions of nucleotides in the *APP* promoter according to Salbaum *et al.* (37). *Bam*HI, *Eco*RI, *Hin*dIII, *Xba* I are restriction enzyme sites abbreviated as B, E, H, and X, respectively. pCLL-BE, pCLL-EB, pCLL-HB, and pCLLXB are different *APP* promoter/hGH constructs. (*B*) The amount of hGH was measured by radioimmunoassay in the medium of AB-1 cells treated (hatched bars) or not treated (open bars) with IL-1β after the transfection with different *APP* promoter/hGH constructs. Ordinate shows hGH in cpm. Each bar represents a mean ± SEM of three experiments. Letters correspond to the restriction sites used in the fusion constructs and identify size and orientation of *APP* promoter fragments with the left letter corresponding to the 5′ end and the right letter corresponding to the 3′ end of the fragment. bp, Base pair.

mRNAs induced by IL-1 was blocked by H7, an inhibitor of protein kinase C (38). Thus, it is possible that this induction may be mediated by the protein kinase C pathway. Interestingly, the topological distribution of the γ isotype of protein kinase C immunoreactivity (38) and the APP mRNA (18) and immunoreactivity (49, 50) is similar in the brain.

The *APP* promoter region responsive to the IL-1 modulation contains one of the two potential AP-1 binding sites. While our data provide evidence for the functionality of one of these sites, it remains to be determined if this site is the only one critical for *APP* gene induction. These results also suggest that the presence of the downstream AP-1 binding site (position −45) is not sufficient for the IL-1-induced changes in the mRNA level of the *APP* gene. However, these data agree with the H7 inhibition studies and argue that IL-1 regulates *APP* gene expression in human endothelial cells by a mechanism mediated by protein kinase C utilizing an AP-1 binding site in the *APP* gene promoter region.

Although the origin of amyloid in Alzheimer disease, Down syndrome, hereditary cerebral hemorrhage (Dutch type), and aging remains unsettled, the vascular endothelial cell model provides a very useful experimental system for the study of the regulation of expression of the *APP* gene. The observation that APP mRNA transcript expression is enhanced by several well-studied factors provides useful information about the functional regulation of the *APP* gene. Thus, the potential involvement of mediators of growth and inflammation in the development of cerebral amyloidosis is an area that warrants further study.

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