

Interleukin-1 β and Glutamate Activate the NF- κ B/Rel Binding Site from the Regulatory Region of the Amyloid Precursor Protein Gene in Primary Neuronal Cultures*

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We originally reported that members of the family of transcription factors NF- κ B/Rel can specifically recognize two identical sequences, referred to as APP κ B sites, which are present in the 5'-regulatory region of the APP gene. Here we show that the APP κ B sites interact specifically with a complex which contains one of the subunits of the family, defined as p50 protein, and that they act as positive modulators of gene transcription in cells of neural origin. Additionally, the nuclear complex specifically binding to the APP κ B sites is constitutively expressed in primary neurons from rat cerebellum and it is up-regulated in response to both the inflammatory cytokine interleukin-1 β (IL-1 β) and the excitatory amino acid glutamate. Since IL-1, whose levels are known to be induced in brain of individuals affected by Alzheimer's disease, and glutamate, are stimuli which have been regarded as major actors on the stage of neurodegenerative processes, we believe our evidence as potentially relevant for understanding the neuropathology associated with Alzheimer's disease.

Alzheimer's disease (AD)¹ is a neurodegenerative disorder characterized by abnormal deposition of extracellular congophilic plaques in the brain. The main constituent of plaques is a 39- to 43-amino acid peptide, named β -amyloid, which is a proteolytic fragment of the amyloid precursor protein (APP) (1). In recent years, most experimental studies have been aimed at understanding the mechanisms of β -amyloid formation and deposition and at identifying potential risk factors which may favor these processes. In particular, APP gene mutations occurring in families with Familial AD have been correlated with disturbance in protein processing, which in turn, may predispose to β amyloid formation (2, 3). However, since APP gene mutations represent a minor percentage of AD cases, it is evident that other mechanisms may exist to account for β -amyloid deposition. In this regard, it should not be underestimated that overexpression of the APP gene may be involved in the pathogenetic mechanisms of amyloid formation, at least in some clinical forms of the disease. Several observations under-

score the potential contribution of the APP gene overexpression to favoring AD neuropathology: (i) the marked accumulation of β -amyloid which correlates with increased levels of APP mRNA in trisomy 21 (Down's syndrome) (4); (ii) the increased levels of APP gene transcripts in specific areas of the AD brains (5–7); (iii) the increased APP mRNA transcription in cultured fibroblasts from the Familial Alzheimer's disease-1 family (8). In addition, post-mitotic neurons which overexpress full-length APP were shown to degenerate and accumulate large amounts of amyloidogenic C-terminal fragments (9). The promoter region of the APP gene (10) has been shown to contain binding sequences for several known transcription factors (11–19). We reported recently (20) that a novel regulatory pathway for APP gene control at the transcriptional level may involve members of the NF- κ B/Rel family. This family of transcription factors is composed of several members (including p50, p52, p65 or RelA, c-Rel, and RelB) that form hetero- and homodimers which are able to trigger signaling from cell membrane to nucleus. In most cell types, these regulators mediate an early pathogen response by coordinately initiating transcription of genes involved in inflammatory, immune, and acute phase responses (21, 22). In particular, we demonstrated that two identical decameric sequences corresponding to 5'-GGGGTTTCAC-3', located in the 5'-regulatory region of the APP gene, are able to specifically bind NF- κ B/Rel proteins (20). The sites, which we referred to as APP κ B sites, interact with a κ B complex which is constitutively expressed in different rat brain areas and in several cell lines, where levels of this DNA binding activity correlates with APP mRNA content. Furthermore, these sites behave as positive modulators of gene transcription when interacting with members of the family of transcription factors containing the p50 subunit. We now report that an APP κ B binding activity, with the same electrophoretic migration properties, affinity, and immunogenicity as the one previously identified from rat brain areas and cell lines of various origin, is present in primary neurons and in neuroblastoma cells. In the attempt to identify the extracellular signals that may contribute to regulate APP gene expression through APP κ B sites, we selected IL-1 and glutamate as good candidates for such a modulatory role. In fact IL-1 is induced in the central nervous system following a variety of insults (23). Moreover, the cytokine is dramatically overexpressed in brains of individuals with AD and Down's syndrome (24) and it induces an increase in APP transcript levels in endothelial (11) and neuronal cells (25). On the other hand, the role of glutamate as a mediator of acute neurodegenerative events is well recognized (26). Here we report that both IL-1 and glutamate can indeed up-regulate the specific APP κ B binding activity in primary neuronal cultures. In transfection experiments the APP κ B site proves to be sufficient to mediate IL-1-induced transcription of a reporter gene.

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¹ The abbreviations used are: AD, Alzheimer's disease; APP, amyloid precursor protein; IL-1 β , interleukin-1 β ; EMSA, electrophoretic mobility shift assay; CAT, chloramphenicol acetyltransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

The contribution of these findings to a better understanding of the pathogenetic mechanisms underlying AD could be relevant. Our working hypothesis is that NF- κ B/Rel proteins can mediate gene expression in response to pathogenic events not only in "periphery," but also in brain. We suggest that the APP gene is one of the κ B-controlled genes in the central nervous system and that this genetic response may potentially contribute to the pathogenesis of AD.

EXPERIMENTAL PROCEDURES

Cell Culture—Primary cultures of cerebellar granule cells were prepared from cerebella of 8-day-old rat pups (Sprague-Dawley), as described previously (27). 12×10^6 cells were plated onto 100-mm Petri dishes coated with 100 μ g/ml L-polylysine and grown in basal medium Eagle's containing 10% fetal calf serum, 2 mM glutamine, 25 mM KCl, and gentamycin (50 μ g/ml). Cells were utilized after 12 days of culturing. The human neuroblastoma SH-SY5Y cell line was grown in minimum essential medium with 10% fetal calf serum, 2 mM glutamine, penicillin (100 units/ml), and streptomycin (100 μ g/ml).

Nuclear Extracts and Electrophoretic Mobility Shift Assay—Nuclear extracts from cell lines and from primary cultures were prepared according to a small scale protocol as in Ref. 28. Protein concentration was assessed by the Bio-Rad Bradford assay according to the manufacturer's instructions. DNA binding reactions were initiated by combining 2–4 μ g of nuclear extracts with 20,000 cpm (0.1 ng) of γ - 32 P-labeled oligonucleotides in $1 \times$ ligation buffer (10 mM Tris-Cl, pH 7.5, 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 10% glycerol) containing 0.5 μ g of poly(dI-dC) in a total volume of 10 μ l. In competition experiments, 2 ng (20-fold molar excess) of unlabeled competitor oligonucleotides were added together with 32 P-labeled probes. Reactions were carried out for 20 min at room temperature, and protein-DNA complexes were resolved on nondenaturing 4% polyacrylamide gels in $1 \times$ Tris glycine-EDTA buffer. Gels were then dried and subjected to autoradiography at room temperature. In supershift experiments, 1 μ l of preimmune or immune serum was incubated with nuclear extracts for 1 h at 4 $^{\circ}$ C, before addition of the other components of the reaction mixture and incubation for an additional 20 min at room temperature. Polyclonal rabbit anti-p50 antibodies were prepared against bacterially produced and affinity purified recombinant mouse p50 as described in Ref. 29, anti-p65 and anti-c-Rel antibodies were from Santa Cruz Biotechnologies.

Synthetic DNA Oligonucleotides—Oligonucleotides were synthesized, together with their complementary strands, using a DNA synthesizer (Applied Biosystem), and purified by denaturing gel electrophoresis. For gel shift analysis double-stranded oligonucleotides were end-labeled with [γ - 32 P]ATP (ICN, specific activity >7,000 Ci/mmol) and T4 polynucleotide kinase (Boehringer Mannheim) to obtain a specific activity of more than 10^8 cpm/ μ g. Sequences were as follows: APP1 (–2257 to –2234 of the APP 5'-regulatory region) 5'-TA-GAGACGGGGTTTCACCGTGTTA-3'; APP2 (–1894 to –1871 of the APP 5'-regulatory region), 5'-AGAGATGGGGTTTCACCATGTTG-3'; mutAPP1, 5'-TAGAGACGctcTTTCACCGTGTTA-3'; oligonucleotide containing the octamer protein binding site from the interleukin-2 gene enhancer region, 5'-TATGTGTAATATGTA AACATTTTGACACC-3'; IL-2 κ B (–212 to –195 of the interleukin-2 gene enhancer region), 5'-CCAAGAGGGATTTCACCTAAATCC-3'. Both strands of the APP oligonucleotide probes have a 5'-TCGA-3' overhang at their 5' end to facilitate cloning.

Plasmid Constructs—Oligonucleotides representing the two putative κ B sites from the APP gene regulatory region, APP1 and APP2, were subcloned into the *Sa*I restriction site of PBLCAT2 (30) to generate APP1 κ BCAT and APP2 κ BCAT constructs, as described in Ref. 20.

Transfection of Cell Lines—SH-SY5Y cells (5×10^5 cells/60-mm dishes) were transiently transfected with 10 μ g of APP1 κ BCAT, APP2 κ BCAT, or PBLCAT2 using the calcium phosphate technique. Four hours after calcium phosphate-DNA addition to cell medium, cells were washed twice with serum-free medium followed by culturing in complete medium. 24 h later, either human recombinant IL-1 β (Boehringer Mannheim), 250 units/ml, or phosphate-buffered saline was added to culture medium. After 16–18 h, cells were collected, washed in phosphate-buffered saline, and lysed by three cycles of freezing and thawing in 250 mM Tris-Cl, pH 7.5. Supernatants obtained by centrifugation for 5 min at 12,000 rpm were sampled to determine protein content and assayed for chloramphenicol acetyltransferase (CAT) activity according to Ref. 31. Transfection efficiency throughout the experiments was monitored by cotransfection with pSV β gal plasmid. Transfection experiments were repeated three times in duplicate with

at least two independent plasmid preparations.

Northern Blot Analysis—Total RNA was extracted with RNAzol from 10×10^6 cerebellar granule cells maintained in culture for 12 days and treated as described, according to the manufacturer's instructions. Aliquots (20 μ g) of RNA were separated on 1.2% agarose-formaldehyde gels and transferred to nylon filters (Hybond N-plus, Amersham). A 1.0-kilobase *Eco*RI fragment of a mouse cDNA clone representing the β -amyloid and the proximal 3'-untranslated region of APP (a kind gift of Dr. G. Forloni), was used to measure β -APP mRNA transcripts. The cDNA probe was labeled using a randomly primed DNA labeling kit from Amersham, according to the manufacturer's instructions. Hybridization conditions were the same as described previously (25). Blots were exposed to x-ray films at –80 $^{\circ}$ C with intensifying screens for the time necessary for the signal to be in the linear range for quantification. GAPDH mRNA was measured in each sample and used as an internal control to normalize for total RNA. Hybridization was quantified by densitometry and expressed as APP/GAPDH ratio.

RESULTS

The DNA Binding Activity Interacting with the APP κ B Site Is Constitutively Present in Primary Neurons—Nuclear extracts were prepared from primary cultures of rat cerebellar granule cells. These cultures consist of a highly homogenous population of neurons. In fact, over 95% of these cells are glutamatergic granule cells, with minimal contamination (less than 3%) by glial and endothelial elements. The experiments were performed on the 12th day of culturing. Extracts were then tested in electrophoretic mobility shift assay (EMSA) for the presence of specific activities able to interact with the APP κ B site. Double-stranded oligonucleotides comprising the APP κ B sites from the 5'-regulatory region of the APP gene and designated as APP1 (–2257 to –2234) and APP2 (–1894 to –1871) were utilized. The results from representative experiments using γ - 32 P-labeled APP1 probe are shown in Fig. 1; comparable results were obtained with the APP2 oligonucleotide (data not shown). The APP1 probe detected a single complex (Fig. 1, *lane 1*) which proved to be a specific DNA binding activity, since it could be displaced by a 20-fold molar excess of the unlabeled APP1 oligonucleotide (*lane 2*), and not by an unrelated oligonucleotide probe (containing an octamer binding site) (*lane 4*) or by a mutated version of the APP1 oligonucleotide with three base changes in the κ B-like core sequence (*lane 5*). Furthermore, to confirm that the nuclear complex was a NF- κ B/Rel-related complex, a competition experiment was performed also with an oligonucleotide containing a well characterized κ B site from the IL-2 gene enhancer region (29). As shown in Fig. 1, *lane 3*, the IL-2 κ B oligonucleotide competed with the APP1 sequence for binding to the nuclear complex.

The APP κ B Binding Complex from Cerebellar Granule Cells Is Immunologically Related to the p50 Subunit of the NF κ B/Rel Family—In order to obtain further information on the subunit composition of the APP κ B complex identified in primary neurons, antibodies specific for several members of the NF- κ B/Rel family were tested for their ability either to interfere with DNA binding or to supershift DNA bound activity. Nuclear extracts were incubated with the γ - 32 P-labeled APP1 probe in the presence of polyclonal antibodies raised against p50, p65, and c-Rel subunit. As shown in Fig. 1, *lane 6*, the p50 antiserum interacted with the APP κ B binding complex causing a supershift of bound probe. No interaction was observed by incubating the extracts with the anti-p65 (*lane 7*) and anti-c-Rel antisera (*lane 8*). Specificity of results was confirmed with preimmune serum or by incubating the antisera with the peptides against which they were raised (not shown). In conclusion, an APP κ B binding activity, with the same affinity properties and immunogenicity as the one previously identified from rat brain areas and cell lines of various origin (20), is also present in primary neurons.

The Neuronal APP κ B Binding Activity Can Be Up-regulated by Interleukin 1 β —We were interested in verifying whether the

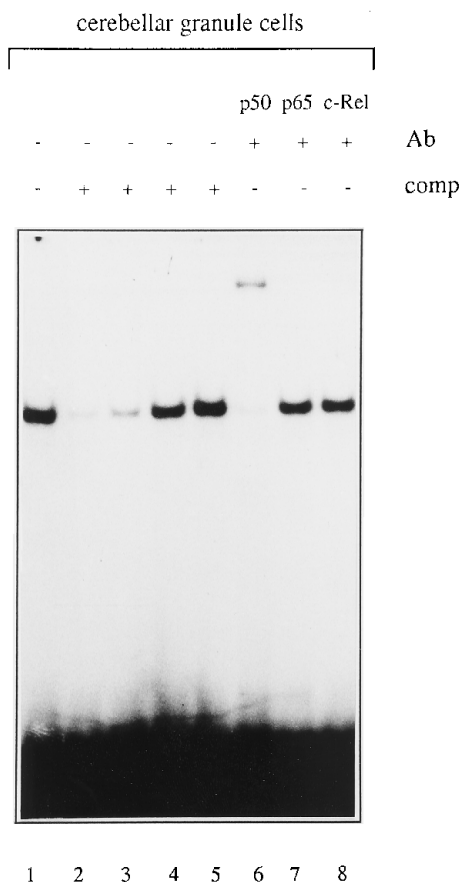


FIG. 1. **Characterization of the APP κ B binding activity in nuclear extracts of cerebellar granule cells.** EMSA was performed using γ - 32 P-labeled APP1 oligonucleotide as a probe. Specificity of DNA-protein interaction was demonstrated by incubating nuclear extracts in absence (lane 1) or in presence of unlabeled APP1 (lane 2), IL-2 κ B (lane 3), an unrelated oligonucleotide containing the binding site for octamer proteins (lane 4), and a mutated version of APP1 (lane 5), as competitors. Molecular composition of the complex was investigated by incubating nuclear extracts in the presence of antibodies raised against p50 (lane 6), p65 (lane 7), and c-Rel (lane 8) subunits.

neuronal APP κ B binding activity could be activated by those extracellular signals that have been proposed to be involved in the chain of events leading to increased APP transcription. Primary cultures of rat cerebellar granule cells were either untreated or treated with increasing concentrations (25, 250, 500 units/ml) of recombinant human IL-1 β for 1 h. Nuclear extracts were prepared and analyzed by EMSA. As shown in Fig. 2, IL-1 β was able to significantly augment APP κ B binding activity compared to untreated cells. The induction was dose-dependent, with maximal activity at a concentration of 250 units/ml. Time dependence of this effect was evaluated as well. Cells were exposed to a dose of 250 units/ml recombinant human IL-1 β for different times (30 min, 1 h, 3 h). Nuclear extracts were prepared and analyzed by gel shift assay with APP1 and APP2 oligonucleotide probes. As shown in Fig. 3A, IL-1 β up-regulation of APP κ B binding activity was already detectable after 15 min, with maximal increase within 1 h. After 3 h of stimulation, APP κ B binding activity was still above controls. In each experiment, quality and quantity of nuclear extract samples were evaluated using, as internal control, an unrelated oligonucleotide probe containing a consensus sequence for octamer-binding proteins, which are unaffected by the cytokine (not shown). Additionally, under the same experimental conditions, exposure of cerebellar granule cells in culture to 250 units/ml IL-1 β resulted in a significant increase in APP transcripts. The increase became detectable as early as

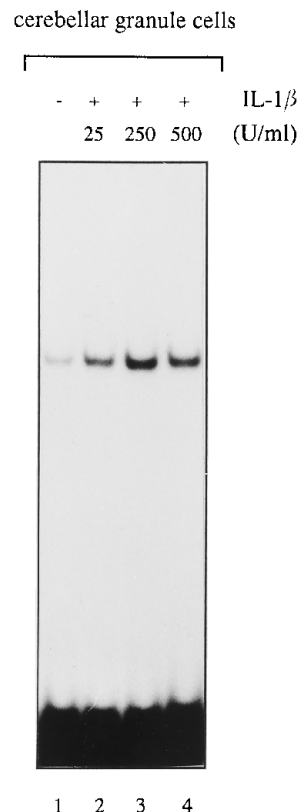


FIG. 2. **Up-regulation of APP κ B binding activity by IL-1 β .** EMSA obtained by incubating 4 μ g of nuclear extracts from rat cerebellar granule cells with γ - 32 P-end labeled APP1 oligonucleotide probe. Cells were untreated (lane 1) or treated with different concentrations of IL-1 β , as indicated in the upper part of the figure (lanes 2–4).

6 h (not shown), but only after 12 h of stimulation APP mRNA content was about 2–3-fold higher than that of untreated cells, as depicted in Fig. 3B.

Transcriptional Activation of the APP κ B Site by IL-1 β in the Human Neuroblastoma Cell Line SH-SY5Y—To directly determine if the APP κ B site is sufficient to confer transcriptional response in cells of neural origin, we transiently transfected human neuroblastoma cell line SH-SY5Y, which is known to express the APP gene, with 10 μ g of the reporter construct APP1 κ BCAT, obtained by cloning the APP1 oligonucleotide fused to a heterologous promoter in the expression vector PBLCAT2 (30). As depicted in Fig. 4A, no difference in reporter gene activity was observed between cells transfected with PBLCAT2 or APP1 κ BCAT vectors. On the contrary, in cells transfected with APP1 κ BCAT, 250 units/ml IL-1 β stimulated a 4-fold increase in promoter activity compared to cells transfected with the control plasmid. Analogous results were obtained with the APP2 κ BCAT reporter vector (obtained by cloning the APP2 oligonucleotide in PBLCAT2, not shown) and in another human neuroblastoma cell line, SK-N-SH (not shown).

The APP κ B-mediated Transcriptional Activity Correlates with Levels of APP κ B Binding Activity—To determine whether IL-1 β induces APP κ B binding activity in SH-SY5Y cells, we performed a gel shift experiment. Nuclear extracts were prepared from neuroblastoma cells which were unstimulated (control) or stimulated with 250 units/ml of IL-1 β for 1, 3, and 6 h. As shown in Fig. 4B, in untreated cells we detected a very low amount of constitutive protein activity binding the APP1 site. A time dependent increase in DNA binding activity was observed in IL-1 β -stimulated cells, with a maximal increase after 3 h of stimulation. Specificity of DNA-protein interaction was confirmed by competition experiments with the unlabeled oligonu-

FIG. 3. Effect of IL-1 β on APP κ B binding activity and APP mRNA levels in primary neuronal cultures of cerebellar granule cells. *A*, gel shift analysis of nuclear extracts incubated with 32 P-labeled APP1 and APP2 oligonucleotides. Cells were either untreated (–) or treated (+) with 250 units/ml recombinant IL-1 β for 15 min, 1 h, 3 h. *B*, APP mRNA levels in untreated (*open bar*) or IL-1 β -treated (*filled bar*) cerebellar granule cells. Quantification of total RNA extracted from neuronal cells after 12 h exposure to IL-1 β (250 units/ml) and hybridized with cDNA probes recognizing total APP and GAPDH mRNA. GAPDH was used as an internal control. Hybridization was quantified by densitometry and expressed as APP/GAPDH ratio. The data are the means \pm S.E. of five determinations. *, $p < 0.01$ versus control.

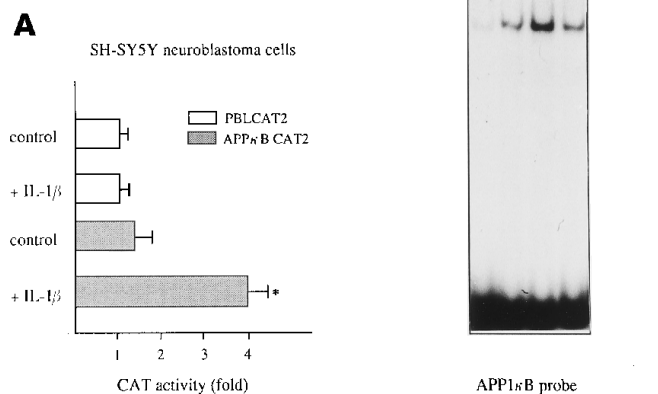
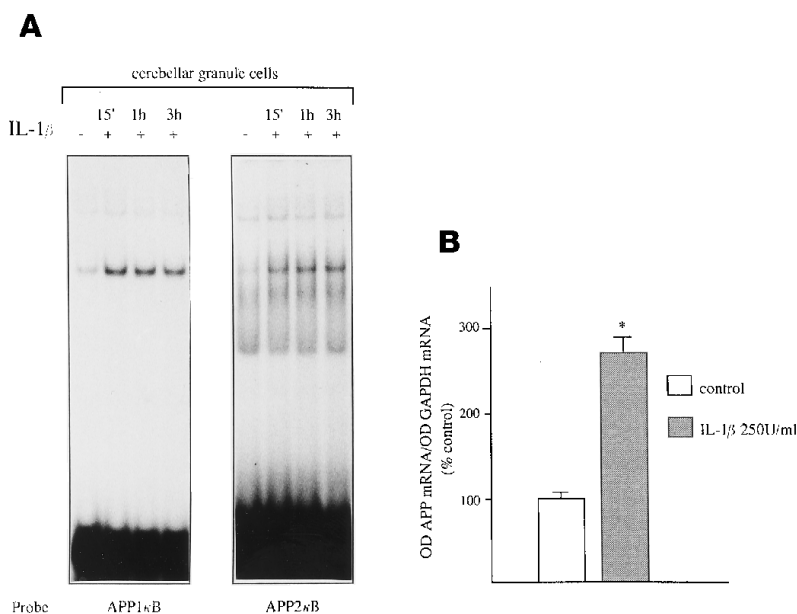


FIG. 4. IL-1 β induced transcriptional activation of an APP κ B driven reporter plasmid and APP κ B binding activity in the neuroblastoma cell line SH-SY5Y. *A*, CAT assay performed on 15 μ g of cytosolic extracts from undifferentiated SH-SY5Y cells transfected with 10 μ g of the reporter plasmid APP1 κ BCAT (obtained by cloning the APP1 oligonucleotide sequence at the *Sa*I site of PBLCAT2) or of the parental plasmid PBLCAT2, either in the absence (control) or presence of 250 units/ml human recombinant IL-1 β (+IL-1 β). CAT activities are expressed as fold induction relative to activity obtained with PBLCAT2. Transfection experiments were repeated three times in duplicate with at least two independent plasmid preparations. *B*, EMSA of nuclear extracts from undifferentiated SH-SY5Y cells untreated (–) or treated (+) with 250 units/ml IL-1 β for different periods of time as indicated in the upper part of the figure. γ - 32 P-labeled APP1 oligonucleotide was used as probe.

cleotide probe (not shown).

The APP κ B Binding Activity Is Modulated by the Excitatory Amino Acid Glutamate—The primary cultures of cerebellar granule cells are a widely recognized model for studying mechanisms of toxicity generated by excitatory amino acids through the activation of the *N*-methyl-D-aspartic acid-type of glutamate receptor (27). Activation of this receptor in the absence of extracellular magnesium permits calcium influx when cells are

stimulated at normal resting membrane potential. In this system, glutamate administered under appropriate experimental conditions (50–200 μ M, 15-min pulse, in absence of magnesium) which are well documented to induce *N*-methyl-D-aspartic acid-receptor activation, results in a significant up-regulation of the APP κ B binding activity (Fig. 5). Glutamate-mediated APP κ B induction became detectable within 30 min and was maximal after 15 min (not shown) when 100 μ M glutamate was utilized as a stimulus.

DISCUSSION

Several clinical and experimental observations underscore the potential contribution of APP gene overexpression to production of the β -amyloid peptide and possibly to the neuropathology associated with AD (4–9). In this regard, it appears important to elucidate the molecular mechanisms of APP gene regulation and identify in the 5'-regulatory region of this gene, *cis*-acting regulatory elements and transcription factors potentially relevant for the control of gene expression. APP overexpression may indeed result from imbalance among regulatory pathways for APP expression (*i.e.* alterations in transcription factors expression or activation) and/or from mutations in the APP gene regulatory region.

We have recently reported the identification of two identical sequences located in the 5'-regulatory region of the APP gene which are specific binding sites for regulatory members of the NF- κ B/Rel family of transcription factors (20). The NF- κ B family is composed of several distinct DNA binding subunits (including p50, p52, or p50B, p65 or RelA, c-Rel, RelB) which can hetero- and homodimerize, thereby forming complexes with distinct cell type distribution, DNA sequence specificity, and transcriptional activity. Properties of these proteins have been most extensively exploited in cells of the immune system or, more in general in periphery, where these regulators mediate an immediate-early pathogen response by coordinately initiating transcription of genes involved in inflammatory, immune, and acute-phase responses (21, 22). On the contrary, until recently the presence and function of NF- κ B/Rel proteins in cells from the nervous system were poorly documented. Some recent elegant reports have suggested that NF- κ B/Rel proteins are likely to participate in normal and pathological brain function (32–35). We now show that an APP κ B binding activity is constitutively present in neuronal primary cultures from rat cerebellum. As far as specificity, affinity, and immunogenicity,

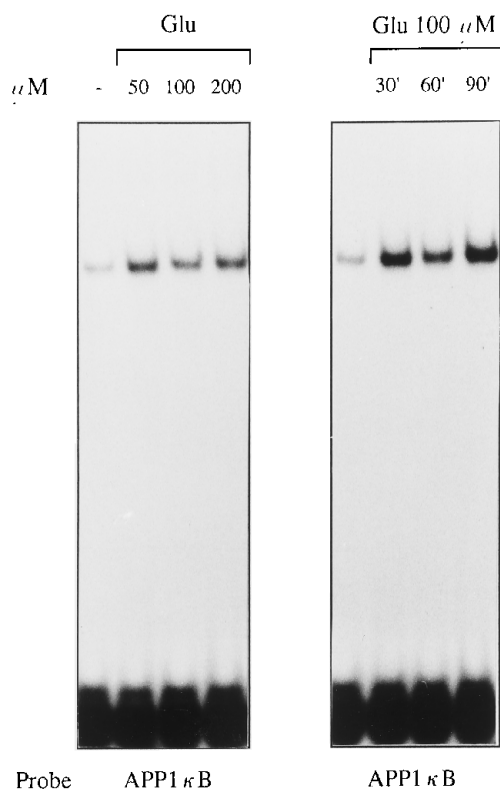


FIG. 5. Dose- and time-dependent induction of the APP κ B binding activity by glutamate. EMSA obtained incubating 4 μ g of nuclear extracts from rat cerebellar granule cells with γ - 32 P-labeled APP1 oligonucleotide probe. *Left panel*, cells were untreated (-) or treated for 1 h with different concentrations of glutamate, as indicated in the upper part of the panel. *Right panel*, cells were untreated (-) or treated with 100 μ M glutamate for different periods of time as indicated in the upper part of the panel.

the nuclear complex is identical to the one previously identified in rat brain, in T lymphocytes and cell lines of various origin (20). As postulated previously, also in neuronal cultures the APP κ B binding complex appears likely to correspond to a p50 homodimer. More important, the nuclear complex, can be modulated in a dose- and temporal-specific manner by IL-1 β and glutamate. When the stimulus is IL-1 β , induction of DNA binding activity at the APP κ B site can be correlated with a parallel increase in transcriptional activity of an APP κ B-driven reporter gene and with increase in APP mRNA levels. Additionally, glutamate can also potently induce the APP κ B binding activity, even at concentrations that are not fully neurotoxic. For glutamate, an analogous correlation between APP κ B binding activity and APP mRNA levels was not performed, since the active concentrations cause neuronal death within the same time frame. Nevertheless, we have indirect evidence that glutamate can result in APP gene expression, since under the same experimental conditions, stimulation of the *N*-methyl-D-aspartic acid receptor causes a significant increase in APP immunoreactivity (36).

These data allow us to hypothesize that two major pathogenic pathways which are likely to contribute to the neuropathology associated with Alzheimer's disease (inflammatory cytokines and excitatory amino acids), although distinct, share a common step represented by activation of a NF- κ B/Rel activity. This event may ultimately result in APP gene overexpression. In this respect it should be further extended by the recent observation, made by Yan and colleagues (37), that NF- κ B activity is significantly augmented in AD brain compared to controls. Transfer of this knowledge to the understanding of the neuropathology of AD, although reductive, could contribute

to open novel perspectives in the way we think about the disease. Our data may indeed support the hypothesis that in AD both the inflammatory cytokine IL-1 β and excitatory amino acid glutamate can contribute to NF- κ B induction and that, in turn, augmented NF- κ B activity can participate in dysregulated expression of the APP gene.

We propose that NF- κ B/Rel proteins may represent the point of convergence of several signaling pathways relevant for initiating or accelerating those events, like inflammatory mediators, excitatory amino acids, and glycated τ formation (37), that ultimately contribute to the process of neuronal dysfunction and degeneration in AD. To this regard, the APP itself, whose gene expression is up-regulated in response to several stressing circumstances including head injury, neurotoxicity, and focal ischemia (38–43) could be viewed as acute-phase protein. We speculate that the APP gene may be one of a set of κ B-site containing genes, coordinately modulated in brain in response to situations that require a defensive reaction. In fact, many of the genes which are induced in periphery during stress conditions are under control of NF- κ B/Rel proteins. Among them it is the serum amyloid A gene, which encodes for another amyloidogenic protein (44).

If APP has to be regarded as a reactive protein, somehow crucial for cellular response to various threatening conditions, how will this response turn out to be deleterious in AD? We have a pragmatic working hypothesis. If induction of APP transcripts, whose original significance is defensive, was inappropriate as far as time (prolonged persistence of the noxa, and/or defect in mechanisms that may restrict/terminate the response), site (different rates of APP expression may occur in various brain areas), or intensity, it could result in augmented production of the β -amyloid. Therefore overexpression of the APP gene should be regarded as a potential risk factor for neuritic plaque formation.

If NF- κ B/Rel proteins represented an integrating point which conveys several pathways potentially contributing to the pathogenesis of AD, molecules that finely modulate their activity could also prevent and/or retard the progression of the disease. More in general, it is a reasonable prospect that this and other studies on the transcriptional control of the APP gene may help to develop novel therapeutic strategies to be applied in AD cases where APP overexpression is highly contributory to occurrence of the disease.

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