

ApoE Synthesis in Human Neuroblastoma Cells

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Received January 17, 1997, accepted for publication August 15, 1997

Apolipoprotein E (apoE) is associated with the two hallmarks of Alzheimer's disease: A β deposits and neurofibrillary tangles. ApoE synthesis was detected in astrocytes by *in situ* hybridization but was not detected in neurons. Nevertheless, different studies on apoE immunoreactivity reported the presence of apoE in neurons of Alzheimer, control, and necrosis pontisubicular brains. In this study, we addressed the question of potential synthesis of apoE in neurons and its possible involvement in or in response to pathological conditions. To this purpose, we have studied human neuronal cell lines (SY 5Y and Kelly cells) originating from neuroblastoma. Using monoclonal and polyclonal antibodies, a 32-kDa band was detected in SY 5Y and Kelly cells, before and after NGF differentiation. Two-dimensional gel electrophoresis analysis showed a typical profile of apoE spots resolved to the exact isoelectric points. By reverse transcription-polymerase chain reaction experiments, we demonstrated the presence of apoE mRNA in these cell lines. SY 5Y cells synthesized the apoE3 variant, whereas Kelly cells expressed both apoE3 and apoE4 isoforms, corroborating the two-dimensional gel results. These results suggested that apoE synthesis could occur in human neuronal cell lines under certain conditions. © 1997 Academic Press

INTRODUCTION

Apolipoprotein E (apoE) is the most abundant apolipoprotein in the human brain, which is the second major site of its synthesis (Elshourbagy *et al.*, 1985). ApoE exists as three major isoforms which differ from one another by the presence of a Cys or an Arg at the 112 and 158 AA positions (Weisgraber *et al.*, 1981). These isoforms are named E2 (Cys 112, Cys 158), E3 (Cys 112, Arg 158), and E4 (Arg 112, Arg 158).

In recent years, many reports have provided evidence of a potential role for apoE in Alzheimer's disease (AD). An increase of apoE mRNA was observed in astrocytes of AD brains (Diedrich *et al.*, 1991). Numerous genetic studies clearly demonstrated a modulation of the apoE effect according to the expressed alleles: the ϵ 4 allele acts as a risk factor (Strittmatter *et al.*, 1993; Saunders *et al.*, 1993; Corder *et al.*, 1993; Mayeux *et al.*, 1993; Poirier *et al.*, 1993), whereas the ϵ 2 allele may have a protective effect

(Chartier-Harlin *et al.*, 1994; Corder *et al.*, 1994). Immunohistochemical studies showed that apoE is present in the two neuropathological abnormalities characteristic of this disease: extracellular amyloid deposits and intracellular neurofibrillary tangles (Namba *et al.*, 1991; Strittmatter *et al.*, 1993). Furthermore, allele ϵ 4 may have an effect on the duration of the disease and on the amyloid deposits (Frisoni *et al.*, 1995; Schmechel *et al.*, 1993).

In vitro studies have shown a possible direct interaction of apoE with the major component of both neuropathological markers of AD: A β peptide, the main component of amyloid deposits (Strittmatter *et al.*, 1993; Wisniewski *et al.*, 1994); and Tau proteins, the main constituent of the paired helical filaments, which are themselves assembled in neurofibrillary tangles (Strittmatter *et al.*, 1994; Huang *et al.*, 1995; Richey *et al.*, 1995; Fleming *et al.*, 1996; Ledesma *et al.*, 1996). These interactions are dependent on the apoE isoform and need the presence of apoE in extracellular space and within neurons. Thus, it is important to determine whether neurons are able to synthesize apoE. To date, apoE synthesis has been detected only in glial cells, in

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particular in astrocytes, by *in situ* hybridization, but it has never been observed in neurons (Diedrich *et al.*, 1991; Poirier *et al.*, 1991). Nevertheless, in addition to detection of apoE in astrocytes, the presence of apoE in human neurons is now supported by numerous immunohistochemical studies. Since the first descriptions by Namba *et al.* (1991) and Strittmatter *et al.* (1993), apoE has been detected in neurons (bearing NTFs or not) from Alzheimer subjects (Han *et al.*, 1994a,b; Benzing & Mufson, 1995; Schmechel *et al.*, 1993), in cortical neurons from normal individuals (Metzger *et al.*, 1996), and in neurons of neonates with pontosubicular neuron necrosis (Arai *et al.*, 1996). Pyramidal neurons seem to be preferentially apoE-immunoreactive and the neuron staining intensity in different cortical layers appears laminar (Schmechel *et al.*, 1996; Metzger *et al.*, 1996). In rodent brains, immunocytochemistry for apoE revealed immunoreactivity in several cellular types, in particular in astrocytes, but not in neurons (Schmechel *et al.*, 1996; Xu *et al.*, 1996). When human apoE is expressed in transgenic mice on an apoE knockout background, the apoE-immunopositive neurons are located in specific cortical layers, as was similarly described for primates, whereas no neurons are immunostained for apoE in wild mice (Xu *et al.*, 1996). ApoE present in the neurons seems to be preferentially located in the cell cytoplasm, as demonstrated by electron microscopy studies (Han *et al.*, 1994a; Xu *et al.*, 1996). The presence of cytoplasmic apoE can be explained either by neuronal synthesis of a cytoplasmic form or by direct insertion into the cytoplasm. The latter happens with some bacterial toxins, in particular with *Pseudomonas* exotoxin A, which binds to the same LRP receptor as apoE-enriched lipoproteins (but without intermediate proteoglycan binding). After endocytosis, it escapes lysosomal degradation by translocating across intracellular membranes into the cytoplasm (review in Krieger & Herz, 1994).

Thus, to understand how apoE could interact with Tau or other cytoskeleton proteins and to determine which mechanisms are implicated, it is important to verify that apoE synthesis can take place in the neurons themselves. To investigate the ability of neurons to synthesize apoE, a cell culture system has been used to study neuronal cells of human origin in the absence of glial cells. In this article, we describe the intracellular presence of apoE in SY 5Y and Kelly cells. Reverse transcription-polymerase chain reaction (RT-PCR) experiments directly proved that these two human neuroblastoma cell lines have the ability to synthesize apoE.

MATERIALS AND METHODS

Cell Cultures

SY 5Y and Kelly cells were respectively maintained in Dulbecco's modified Eagle's medium and RPMI 1640 (Gibco BRL) supplemented with 10% fetal calf serum (Boehringer Mannheim). Differentiation of SY 5Y cells was performed with NGF treatment for 4 or 8 days, in the absence of serum, as described elsewhere (Dupont-Wallois *et al.*, 1995).

Protein Extraction and Western Blot Analysis

The cells were harvested at 4°C and collected by centrifugation. Laemmli sample buffer (5% SDS, 0.25% dithiothreitol) was added to the cell pellet. Human or fetal calf serum ($\pm 20 \mu\text{g}$) was directly diluted in the Laemmli buffer. Human serum came from a control subject. Brain tissue was homogenized in Laemmli buffer (1% wt/vol). The samples were denatured by heating at 100°C for 10 min as described in Dupont-Wallois *et al.* (1995).

After electrophoresis and transfer onto nitrocellulose, the Western blotting method was used to determine the presence of different proteins. The presence of Tau proteins was examined using M19G serum (Sautière *et al.*, 1994; Dupont-Wallois *et al.*, 1995). The polyclonal antiserum 345 recognizes neurofilament subunits NF-L, NF-M, and NF-H, whereas SMI31 monoclonal recognizes a phosphorylated epitope in the carboxy-terminal domain of NF-M and NF-H (Sternberger & Sternberger, 1983). Monoclonal GF5 antibody was specific for the glial fibrillary acidic protein (GFAP) (David *et al.*, 1994). Rabbit polyclonal antibody to NSE (neuron-specific enolase) was purchased from Affiniti (TEBU, France). The presence of apoE was investigated using two distinct antibodies: a rabbit polyclonal antiserum raised against the whole apoE and the EO1 monoclonal antibody for which the epitope is located on the amino-terminal part of apoE (Leroy *et al.*, 1988). Both apoE antibodies were a generous gift from Dr. J. C. Fruchart, and EO1 in particular was used in numerous works (Buée *et al.*, 1996; Lefranc *et al.*, 1996; Gracia *et al.*, 1994). The specificity of apoE binding was checked in simultaneous incubations of EO1 antibodies (1/5000) and apoE4 recombinant protein (0.5 $\mu\text{g}/3 \text{ ml}$) purchased from Panvera (Medgene Science S.A.).

Two-Dimensional Electrophoresis

After washing with PBS buffer, the cells were collected by centrifugation, resuspended in Laemmli

buffer, and heat-treated at 100°C for 5 min. Two-dimensional gel electrophoresis was performed as described in Sergeant *et al.* (1997). Briefly, for the first dimension, samples were adjusted to a final concentration of 8 M urea and 2% Triton X-100 and were laid onto an isoelectric focusing gel containing 4% (wt/vol) acrylamide and 2.5% (wt/vol) bisacrylamide, 9.5 mol/L urea, 2% (vol/vol) Triton X-100, 4% (vol/vol) pH 3–10 Pharmalytes, and 1% (vol/vol) pH 4–6.5 Pharmalytes (Pharmacia). The second dimension was performed on a 10–20% gradient SDS-PAGE.

Culture Medium Delipidation

Two milliliters of culture medium was centrifuged at 10,000g for 15 min at 4°C. The pellet was resuspended in ether and frozen at –20°C for 2 h. After a second centrifugation, the pellet was resuspended in 70 µl of Laemmli buffer and heat-denatured. The sample was analyzed by dot-blot experiments.

Analysis of Different Transcript Expression

Total RNA was extracted from cells with the RNeasy Lysis Buffer method (Qiagen/Biotecx) according to the manufacturer's instructions. The yield and the quality of RNA preparations were determined by spectrophotometry. Analysis of the transcripts was performed using RT-PCR.

The primers used in this study were chosen for analysis of the cellular expression of apoE and GFAP. The primers used for the detection of GFAP cDNA were described in Reeves *et al.* (1989), and those for apoE cDNA were described in Pérez-Tur *et al.* (1995). A 1.5- to 2-µg amount of each RNA sample was reverse-transcribed with the Mu-MLV reverse transcriptase (Gibco BRL) using the antisense-specific primer. As the primers used for apoE cDNA detection are located within the same exon of the gene (exon 4, described in Pérez-Tur *et al.* (1995)), total cellular RNA was first treated with DNase I to ensure the absence of genomic DNA contamination (Eurogentec) and then used for RT-PCR experiments. To check the efficiency of the DNase treatment, positive control PCR experiments were performed directly using the treated total RNA samples for PCR amplification by omitting the reverse transcription step. The synthesized cDNA was then subjected to 30 cycles of amplification using the ready sense primer for each amplification. In each RT-PCR assay, a negative control was performed by replacing total RNA with water. The PCR products were analyzed on 2% (wt/vol) agarose gels and visualized by ethidium bromide.

ApoE Genotype

Exon 4 of the *APOE* gene was amplified by PCR from genomic DNA as described in Pérez-Tur *et al.* (1995). The PCR products were specifically digested with restriction enzyme *CfoI* and subjected to electrophoresis on a 10% nondenaturing polyacrylamide gel. The cell genotype was identified according to the digested product sizes.

RESULTS

Neuronal Marker Expression

SY 5Y and Kelly cells are described as neuronal-type cells of tumoral origin (Biedler *et al.*, 1973; Schwab *et al.*, 1983). In preliminary experiments, we checked that both cells always expressed neuronal markers, such as neurofilaments and NSE, and not glial proteins, such as GFAP.

On Western blots, the three species of neurofilaments (NF-H, 200 kDa; NF-M, 160 kDa; and NF-L, 70 kDa) were revealed in SY 5Y and Kelly cellular extracts. SMI31 monoclonal antibody detected two bands of 200 and 160 kDa corresponding to NF-H and NF-M, respectively, whereas the polyclonal 345 antibody detected three species: NF-H, NF-M, and NF-L (Fig. 1A). As expected, neurofilament detection increased after cellular differentiation, as shown in Fig. 1A, using the monoclonal SMI31.

The NSE was also immunodetected by Western blotting before and after cellular differentiation (data not shown).

No trace of GFAP was detected either by Western blotting (Fig. 1B) or by RT-PCR in cells (Fig. 1C). The GFAP detection from brain homogenates was used as positive control for Western blotting and RT-PCRs.

Intracellular Presence of ApoE

Western blotting was used to investigate the presence of apoE in the cellular extracts. Well-characterized monoclonal and polyclonal antibodies detected bands of equal apparent molecular weights in SY 5Y and Kelly cell extracts (Fig. 2A). The same band was also detected in SY 5Y cells differentiated by 4 (Fig. 2A) or 8 days (not shown) of NGF treatment. However, this band showed a lower molecular weight (32 kDa) than apoE from brain tissue homogenates (35 kDa) or human serum (34 kDa). Anti-apoE antibodies were also tested on fetal calf serum and no immunoreactive band could be observed (Fig. 2A).

The specificity of the recognition was proved by the

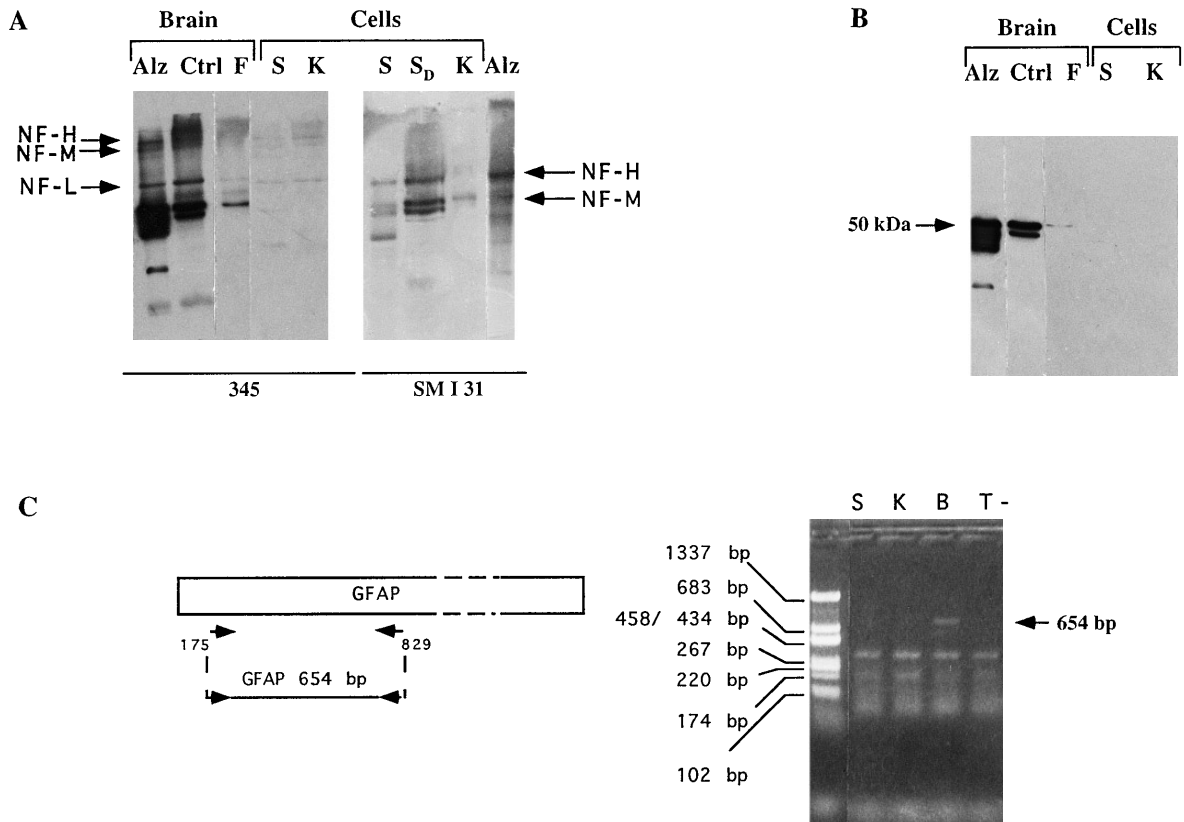


FIG. 1. Neuronal and glial markers analysis. (A) Neuronal markers: presence of the three neurofilament proteins in SY 5Y (S), NGF-differentiated SY 5Y (S_D), and Kelly (K) cell extracts as detected by Western blotting with polyclonal antiserum 345, which recognizes the NF-L, NF-M, and NF-H subunits, or with monoclonal SMI31, which reacts with extensively phosphorylated NF-H and, to a lesser extent, with NF-M. In cells, the three subunits of NF were seen with the polyclonal antibodies 345, whereas NF-H and NF-M are detected with SMI31. Note an increase in SMI31 detection after cellular differentiation (S_D). Homogenates from Alzheimer (Alz), control (Ctrl), and fetal (F) brains were used as positive control. (B) Glial marker: Western blotting with monoclonal GF5 antibody. GFAP was detected only in Alzheimer (Alz), control (Ctrl), and Fetal (F) brain homogenates, not in SY 5Y (S) or Kelly (K) cell extracts. (C) RT-PCR experiments for GFAP detection. No amplification products were obtained with cellular RNAs, whereas a band of the correct size was observed with brain RNA preparations.

absence of the cellular 32-kDa band in the simultaneous presence of EO1 monoclonal antibodies and apoE4 recombinant purchased from Panvera (Medgene Science S.A.) as described under Materials and Methods (Fig. 2B). However, the extinction of the apoE signal was observed only with a narrow ratio of apoE4/antibodies.

The last argument in favor of a cellular apoE presence came from two-dimensional electrophoresis (Fig. 2D). We compared apoE profiles using the polyclonal serum on human brain homogenates for which the genotype was $\epsilon 3/\epsilon 3$ or $\epsilon 3/\epsilon 4$ and on Kelly cells. For the three samples, apoE was detected in a gel region corresponding to pI 5.25–5.45 and of molecular weight around 34–38 kDa, but the pattern differed in the function of the apoE genotype, the degree of sialylation, and the degree of glycosylation as described in

Zannis *et al.* (1981, 1986), Zanni *et al.* (1989), and Visviskis *et al.* (1986). We observed a clear resemblance of cellular and $\epsilon 3/\epsilon 4$ brain apoE patterns: we could distinguish the apoE3 isoform (spot 2) from the apoE4 isoform (spot 1), and these proteins from their sialylated products (spots 3, 4, and 5). Note that $\epsilon 3/\epsilon 3$ brain apoE was different because of the absence of the apoE4 variant.

In addition to the 32-kDa band, some bands of higher molecular weight were faintly detected in the different cellular extracts (not shown). The bands of higher molecular weight were also detected in brain homogenates, in particular with EO1 antibodies. Attempts to correlate these bands with the apoE genotype of the brain samples or with the disease were unsuccessful (data not shown).

The presence of secreted apoE in the culture me-

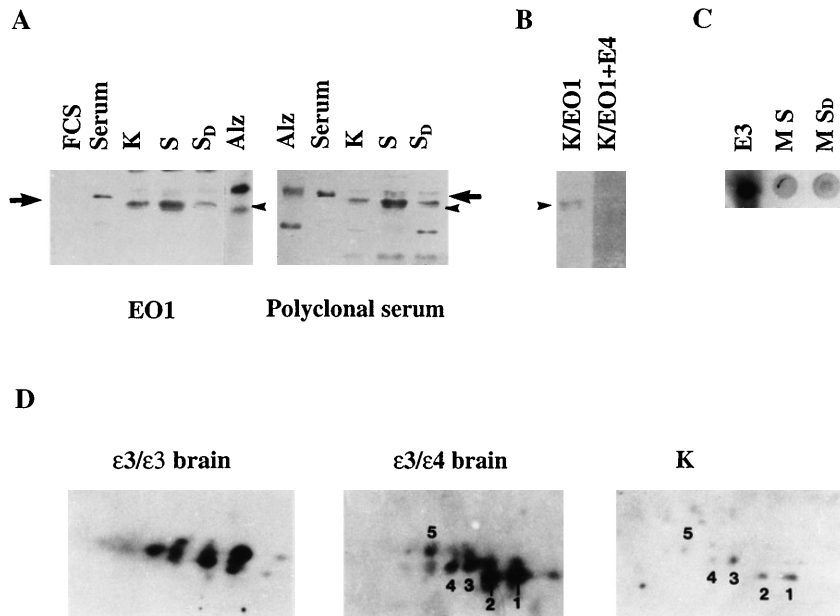


FIG. 2. Analysis of apoE expression in neuroblastoma cells. (A) Analysis of apoE expression in neuroblastoma cells (SY 5Y, S; NGF-differentiated SY 5Y, S_D; Kelly, K) by Western blotting with both monoclonal antibody EO-1 and polyclonal serum. Alzheimer brain (Alz), human serum (serum), and fetal calf serum (FCS) were also analyzed with both antibodies. The arrowheads show the immunoreactive bands corresponding to cell apoE, and the arrows indicate the apoE of human serum or Alzheimer brain homogenate. (B) Specificity of the EO1 antibody was checked by comparison of Western blots revealed using the antibody alone (K/EO1) or previously incubated with apoE4 recombinant protein (K/EO1 + E4). Note the disappearance of the 32-kDa band when antibody was saturated with apoE4. (C) Dot-blot detection of apoE secreted into the cellular culture medium. E3 corresponded to an apoE3 recombinant sample (80 ng), and MS and MS_D to SY 5Y- and NGF-differentiated SY 5Y-delipidated mediums, respectively. (D) Two-dimensional gel electrophoretic patterns of apoE from brain genotyped $\epsilon 3/\epsilon 3$, brain genotyped $\epsilon 4/\epsilon 4$, and Kelly cells. Only the area of the gel in the vicinity of apoE is presented. Spot 1 corresponds to the apoE4 isoform, Spot 2 corresponds to the apoE3 isoform, and spots 4, 5, and 6 correspond to the sialylated products.

dium was investigated by dot-blot experiments. Only dot-blot experiments performed after sample delipidation suggested a possible but faint apoE secretion in the medium (Fig. 2C).

Cellular Synthesis of ApoE

To demonstrate that human neuroblastoma cells are able to synthesize apoE, the apoE transcripts were investigated by specific amplification of exon 4 by RT-PCR (Fig. 3B). A band of 244 bp, similar to the expected size, was amplified with each total cellular mRNA. The absence of the amplified product in the control experiments and the restriction analysis of the PCR products (not shown) indicated that the RT-PCR-amplified products resulted from the apoE transcripts.

Identification of the Isoforms Expressed by Each Line

We further investigated which apoE variants were expressed in neuroblastoma cell lines. First, the APOE

genotype was determined for each cell line as described under Material and Methods. After genomic APOE amplification, the analysis of the *CfoI*-digested products showed that the genotype of SY 5Y was $\epsilon 3/\epsilon 3$ (characterized by the 91- and 48-bp bands) whereas that of Kelly was $\epsilon 3/\epsilon 4$ (characterized by the 91-, 72-, and 48-bp bands) (Fig. 3C). The nature of the apoE transcripts of Kelly and SY 5Y had to be investigated by the RT-PCR product analysis after digestion by the *CfoI* enzyme. The result of the experiment on Kelly cDNA is shown in Fig. 3D. The presence of 91-, 72-, and 48-bp fragments agreed with the expression of both apoE3 and apoE4 mRNA in Kelly neuroblastoma cells. A similar analysis of SY 5Y mRNAs (RT-PCR and *CfoI* digestion) confirmed the expression of only the apoE3 variant by the SY 5Y cells (not shown).

DISCUSSION

This report is the first demonstration of apoE synthesis by neuronal-type cells. The two SY 5Y and Kelly cell

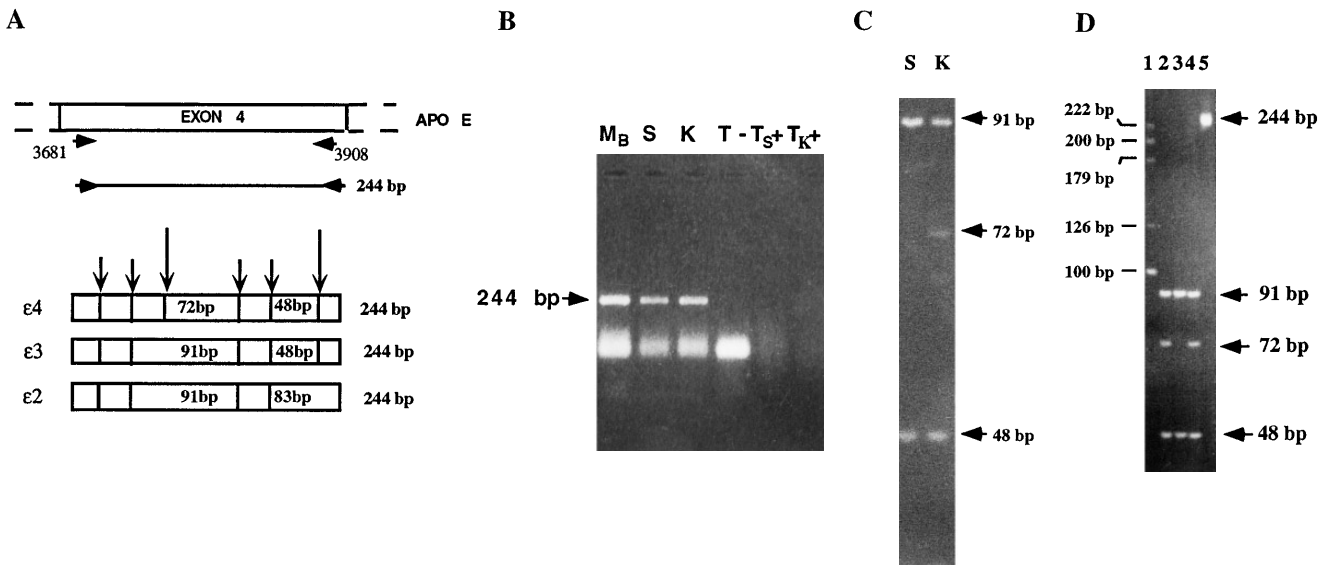


FIG. 3. Analysis of apoE transcripts in both SY 5Y and Kelly cells. (A) Schematic draft representing the amplified polymorphic area (exon 4) of apoE by RT-PCR. (Bottom) Representation of the different *CfoI* restriction sites located on exon 4 of the three *APOE* alleles. Small and large arrows show the common and polymorphic *CfoI* restriction sites, respectively. The specific product sizes are annotated on the schematic draft. (B) ApoE synthesis investigation by RT-PCR in SY 5Y (S) and Kelly (K) cells. The cellular RT-PCR products are loaded on a 2% (w/v) agarose gel and their migration is compared to that of ApoE RT-PCR product amplified from mRNAs of adult brain (M_B). T- corresponds to the negative control performed by replacing cellular RNA by water; T_S^+ and T_K^+ correspond to direct amplification on SY 5Y and Kelly RNAs, respectively, by omitting the reverse transcription step as described under Materials and Methods. (C) SY 5Y (S) and Kelly (K) cell genotype analysis. The cell genotype was identified according to the *CfoI*-digested product sizes analyzed on a 10% nondenaturing polyacrylamide gel as described under Materials and Methods. (D) Identification of the Kelly apoE transcripts. Kelly RT-PCR products (244 bp) were digested by restriction enzyme *CfoI* and compared to the ϵ_3/ϵ_4 and ϵ_3/ϵ_3 size markers after migration through a 10% nondenaturing polyacrylamide gel. ϵ_3/ϵ_4 and ϵ_3/ϵ_3 markers corresponded to the *CfoI*-digested ApoE cDNA from human brains genotyped ϵ_3/ϵ_4 and ϵ_3/ϵ_3 , respectively. Lane 1, mix of 100-bp ladder and pGEM DNA marker (Promega); lane 2, ϵ_3/ϵ_4 marker size; lane 3, ϵ_3/ϵ_3 marker size; lane 4, amplified products from Kelly total RNA digested by *CfoI*; lane 5, RT-PCR product before digestion.

lines used for our study were derived from a human neuroblastoma. SY 5Y cells have been widely described in the literature. These cells are an adrenergic cell line established from human neuroblastoma cells, SKNSH (Biedler *et al.*, 1973), and many of their neuronal features have been reported (West *et al.*, 1977; Biedler *et al.*, 1978; Ammer & Schulz, 1994). They can be differentiated by NGF treatment. Kelly cells are less well known but were described for the first time by Schwab *et al.* (1983). In this study, to confirm the neuronal feature of SY 5Y and Kelly cells, we have chosen to check for the presence of neuronal markers such as neurofilaments and NSE and the lack of glial markers such as GFAP. The three neurofilament subunits (NF-L, NF-M, and NF-H) were effectively detected by polyclonal and monoclonal antibodies in the cells. NF immunodetection was amplified after SY 5Y NGF treatment with monoclonal antibody SMI31, which binds to phosphorylated neurofilaments (Sternberger & Sternberger, 1983). The initial appearance of NF proteins is known to occur early during neuronal

development *in vivo* (Cochard & Paulin, 1984; Carden *et al.*, 1987). Therefore, the presence of NF and NSE and the lack of GFAP confirmed the neuronal type of both cell lines.

The presence of intracellular apoE in Kelly and SY 5Y cell lines, differentiated or not, was first investigated using Western blotting. The specificity of the detected band was checked (1) by the use of two distinct antibodies; (2) by inhibition of cellular apoE detection in simultaneous incubations of antibodies and recombinant apoE; and (3) by two-dimensional gel electrophoresis. Two-dimensional electrophoresis experiments allowed us to assume that (1) the 32-kDa band revealed by the anti-apoE antibodies used in this study actually corresponded to apo E since the immunodetected spots have isoelectric points which exactly correspond to those given by the Swiss-2DPage database (Sanchez *et al.*, 1995), and (2) the apo E sialylation occurred in neuroblastoma cells. However, the apparent molecular weight of apoE in SDS-polyacrylamide gel in our cell cultures was slightly different from that

of human serum. Several factors are known to influence electrophoretic migration, and thus several explanations are possible: a difference in the degree of oxidation, the substitution of one of the amino acids, or a difference in posttranslational modifications, excluding sialylation, as most of the apoE in the serum (Zannis & Breslow, 1981) or in the cells (Fig. 2D) does not seem to be sialylated. Thus, this difference in the molecular weight probably indicates a subtle difference in the molecular structure.

The cellular apoE probably corresponded to the cell-synthesized apoE and not to an uptake from the culture medium. First, in our experiments, apoE was never detected in the fetal calf serum. This result agrees with the fact that this serum is known to be poor in lipoproteins. Second, apoE is present after 4 or 8 days of cell culture in the absence of serum, during the differentiation experiments. In a model of apoE uptake, the extracellular apoE internalized was completely degraded after 24 h of cell culture in the absence of exogenous apoE (Jensen *et al.*, 1994). Third, the phenotype of Kelly cells, determined by two-dimensional gel analysis, corresponded to the transcripts, determined by RT-PCR experiments.

In addition, the capacity of the human neuroblastoma cells to synthesize apoE was demonstrated by the detection of apoE mRNA in the cells, using RT-PCR. Together, our data strongly support *in vivo* apoE synthesis by neuronal cells in culture, but, of course, this does not mean that all brain neurons are able to synthesize apoE *in vivo* or at all stages of their life. Indeed, apoE expression by neuronal cells in the nervous tissues is probably regulated in a different way than in an *in vitro* cellular model. This could explain why apoE synthesis was never detected in neurons although neuronal apoE was detected by different authors in human brain slices using immunohistochemical methods (Namba *et al.*, 1991; Strittmatter *et al.*, 1993; Han *et al.*, 1994a,b; Benzing & Mufson, 1995; Schmechel *et al.*, 1993; Metzger *et al.*, 1996; Arai *et al.*, 1996). Nevertheless, the possibility that some pathologies might restore or exacerbate the ability of the neuron to synthesize apoE proteins cannot be excluded. An effect of pathology on apoE expression has already been reported in AD and in a sciatic nerve injury model (Diedrich *et al.*, 1991; Muller *et al.*, 1985).

In addition to the demonstration of apoE synthesis by neuronal-type cells, an interesting outcome of this study came from the difference in apoE genotypes of these cells: SY 5Y cells expressed apoE3 protein, whereas Kelly cells synthesized both apoE3 and apoE4.

The analysis of a third human neuroblastoma cell

line, LA-N-2 cells, characterized previously as a cholinergic neuronal cell line (Seeger *et al.*, 1977; Singh *et al.*, 1990), confirmed that the neuronal synthesis of apoE was not restricted to one neuroblastoma cell type but could be representative of a more general phenomenon (not shown). These last cells were genotyped $\epsilon 3/\epsilon 3$ (not shown), like the SY 5Y cells.

In conclusion, this article is the first report of apoE synthesis by neuronal-type cells. This result may be important in understanding AD mechanisms. Neuronal apoE expression may differ during development, aging, or pathology. In the present biological system, we show that a physiological level of apoE is compatible with normal development of the cells. Such cell lines (genotyped $\epsilon 3/\epsilon 3$ for SY 5Y cells or $\epsilon 3/\epsilon 4$ for Kelly cells) are likely to be of great interest in studies of the role of apoE in a cellular model of neurodegeneration.

ACKNOWLEDGMENTS

This work was supported by the Institut National de la Santé et de la Recherche Médicale and the Institut de Recherches Servier. C.S. is a recipient of a grant from Servier and N.S. is the recipient of a grant from the association France Alzheimer. We thank Dr. J. C. Beauvillain, Dr. L. Buée, and D. Lefranc for helpful discussions. We are very grateful to Drs. J. C. Fruchart and H. Parra for both monoclonal and polyclonal antibodies directed against apoE.

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