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Differential APP gene expression in rat cerebral cortex, meninges, and primary astroglial, microglial and neuronal cultures

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Differential amyloid precursor protein (APP) gene expression was investigated in primary cultures of astrocytes, neurons and microglia from neonatal rat cerebral cortex as well as in meninges, and young and adult cerebral cortex tissues in order to define the possible contribution of individual CNS cell types in βAP deposition. Meninges and neurons contained higher levels of total APP mRNA than glial cells and APP_{695} mRNA was abundant in neurons while glial cells and meninges contained higher levels of KPI-containing mRNAs. These results demonstrate cell-specific transcriptional and post-transcriptional regulation of APP gene expression in CNS cell types. In addition, the steady-state level of APPs in each cell type did not reflect mRNA levels indicating translational or post-translational regulation.

APP gene expression; Central nervous system; Microglia; Astrocyte; Neuron

1. INTRODUCTION

The amyloid deposits of Alzheimer's disease (AD) contain a 3.9-4.2 kDa peptide, the β-amyloid peptide (βAP) [1-3] which is a cleavage product of the amyloid precursor proteins (APPs). Alternative splicing of the APP transcript gives rise to various proteins (APP_{563}, APP_{714}, APP_{751}, APP_{770}), all of which contain a sequence homologous to the Kunitz protease inhibitor (KPI) with the exception of APP_{695}. Three of them, APP_{695}, APP_{751}, and APP_{770}, are highly but differentially expressed in most cells [4-11] whereas APP_{563} and APP_{714} are expressed at lower levels [10-12].

βAP deposition in cortical senile plaques of AD has been proposed to arise from aberrant proteolytic processing of APP [13,14]. The invariable presence of βAP deposits in older patients with Down's syndrome [15,16] and the presence of higher levels of APP mRNA in Down's brains [7] suggest that over-expression of APP may lead to βAP formation. If over-expression of APP leads to βAP deposition, it is imperative to characterize APP gene expression in individual cell types of the CNS. High levels of APP mRNA and protein detected by in situ hybridization and immunocytochemistry suggest that βAP in the parenchymal amyloid deposits originates in neurons [17-24]. The close association of microglial cells with senile plaques has led to the hypothesis that microglia are the producers and processors of βAP [25,26] although it has also been suggested that amyloid deposition precedes microglial involvement [27-29]. Astrocytes, which appear in high numbers and in close proximity to senile plaques [30,31] and have been shown to have elevated levels of APP expression in lesioned brains [32] could also play a role in βAP deposition. In addition, the presence of amyloid in leptomeningeal and cortical vessels suggests vascular tissues may contribute to the formation of βAP [33,34].

It is known that APP_{695} mRNA is predominantly expressed in the brain whereas KPI-containing APP mRNAs are more abundant in peripheral tissues [10,11,35,36]. Quantitative analysis of APP gene expression has so far been done on whole tissues [11,37-39] or regions of the brain enriched in specific cell types [10]. In cultures, APP gene expression assessed qualitatively by S1 nuclease analysis of mouse microglia, astrocytes and neurons, has revealed predominant expression of APP_{695} mRNA in neurons and KPI-containing APP mRNAs in glial cells [39]. Rat type I astrocytes have been reported to contain only non-KPI containing forms of APP [40]. Yet, precise information on the regulation of APP gene expression in individual cell types of the CNS, as well as accurate quantitation of their relative levels of expression, is still needed in order to evaluate their possible contribution to βAP deposition.

In this study, we have quantitated differential APP gene expression in rat cerebral cortex, meninges and primary cultures of cortical neurons, astrocytes and microglia by Northern blots, slot blots, ribonuclease protection assay (RPA) and immunoblots. Primary cell cultures offer advantages in evaluating APP gene expression since the homogeneity of the cell population can be assessed and levels of total as well as individual APP

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transcripts can be reliably determined. The rat cerebral cortex was used as it conveniently provides a sufficient number of cells to pursue these experiments. Regulation of APP gene expression in the rat is likely to be closely related to that of the human since there is 97% homology in their cDNAs [17]. In addition, the human APP promoter is active in rat PC12 cells [41] and shows neuron specific expression in a transgenic mouse [42]. Therefore, information derived from these studies will provide the basis for future studies using human cell cultures.

2. MATERIALS AND METHODS

2.1. *Tissues and cell cultures*

Cerebral cortex was dissected from newborn, 4-day-old or adult rats sacrificed by decapitation. The meninges were peeled off the cortex and rinsed before use for RNA and protein analysis. The cerebral cortex was then frozen for further studies or used for cell cultures which were carried out according to McCarthy and DeVeillis [43] with some modifications (R. Miller, CWRU, Cleveland, OH). The newborn rat cerebral cortex was minced and cells were dissociated in 0.05% trypsin type III (Sigma, St. Louis, MO) for 20 min at 37°C followed by trituration in 4 Kunitz unit/ml DNaseI (Sigma). Cells were plated in 10% FBS/DMEM (Hyclone Lab. Logan, Utah) on poly-L-lysine-coated Falcon 3024 or 3045 flasks.

Astrocytes were obtained by shaking the flasks vigorously 4–6 h after plating. The astrocytes adhere strongly to the flask and all other cell types are removed in the medium. Neurons were recovered in the medium after very gentle shaking of the cortical cultures 4–6 h after plating and were replated on poly-L-lysine-covered flasks. Microglial cells were detached by shaking cortical cultures at 10 days in vitro (DIV) on an orbital shaker for 1 h at 180 rpm [44]. The medium containing the microglial cells was transferred to poly-L-lysine-coated flasks for 1 h. Microglial cells adhere to the flask during this time and other cell types and debris can be removed with the medium.

2.2. *Characterization of the cell cultures*

The purity of astroglial and neuronal cultures was determined by immunofluorescence with polyclonal antibodies to glial fibrillary acidic protein (GFAP) [45] and the monoclonal antibody 1.2.1 to neurofilament proteins [46], respectively, following fixation in 2% paraformaldehyde. Microglial cultures were assessed for their ability to ingest AcLDL-DiI (acetylated low density lipoprotein labeled with 1,1′-dioctadecyl 1-1-3,3,3′,3′-tetramethyl indocarbocyanine perchlorate [47]) according to the manufacturer's instructions (Biomedical Tech. Inc., MA). Contaminating fibroblasts were identified with antibodies to rat fibronectin (Calbiochem Corp., LaJolla, CA). Oligodendroglia were distinguished by their morphological appearance [48] and their lack of immunoreactivity with the above described antibodies. Immunofluorescence and phase contrast were observed with a Leitz Dialux microscope equipped with epifluorescence illumination.

2.3. *RNA analysis*

Total cellular RNA was extracted as described by Chomczynski and Sacchi [49]. RNA yield was determined by spectrophotometry [50]. Northern blot analysis was carried out using formaldehyde as a denaturant [51]; the RNA was transferred to nitrocellulose and hybridized in formamide at 42°C [52]. The APP probe was the 420 bp *Pvu*II/*Eco*RI fragment of APP_{695} cDNA labeled by random priming [53] at $1.0–1.5 \times 10^9$ cpm/μg DNA. The β-actin probe was a 300 bp fragment obtained by PCR amplification of reverse transcribed rat brain mRNA.

Slot blots were carried out according to Cheley and Anderson [54]. Each slot blot was probed with the 1.056 kb *Eco*RI fragment of APP_{695} or β-actin cDNA probes as described above. The RNA on slot blot autoradiograms was quantitated by densitometric scanning with the LKB Ultroscan XL. The level of APP mRNA was expressed as peak area of APP mRNA/peak area actin mRNA for 1 μg of total RNA and the results from each sample expressed relative to that of the cortex. In order to assure that β-actin was appropriate to normalize the amount of APP mRNA, all slot blots were reprobed with a 6 S rRNA PCR-amplified probe. The levels of 6 S rRNA paralleled those of β-actin.

Ribonuclease protection assays (RPA) were carried out using an RNA probe transcribed from pBluescript KS– carrying a fragment of human APP_{770} cDNA (nucleotides 642–1089; numbering according to Kitaguchi et al. [55]). The RNA probe protects 440 nucleotides in APP_{770}, 386 nucleotides in APP_{751} (and APP_{563}), 223 nucleotides in APP_{695} and two fragments of 223 and 56 nucleotides in APP_{714}. Neither APP_{714} mRNA, which has been observed at very low levels in rat brain [11] nor APP_{563} mRNA which was not detectable in the newborn rat cortex by PCR (results not shown) are likely to be represented in the RPA results; therefore the three bands observed are probably due to APP_{695}, APP_{751} and APP_{770} mRNAs, although the 2.7 kb APP mRNA which contains the KPI sequence could be part of APP_{751} or APP_{770} mRNA. The RPA was carried out with the RPA-ribonuclease protection assay kit (Ambion Inc., Austin, TX) according to the manufacturer's instructions. The ratio of each APP mRNA was determined by densitometric scanning of the autoradiograms and corrections were made to account for the ^{32}P-dCTP content of the probe. The results are expressed as peak area of a specific mRNA/total peak area for all three APP mRNAs. Statistical difference between two samples was evaluated by Student's 2-tailed unpaired *t*-test.

2.4. *Protein analysis*

Cellular proteins were obtained by homogenization of cells in 0.1 M Tris-HCl, pH 7.4, and centrifugation at 16 000×*g* for 30 min at 4°C. The supernatant which did not contain any APP immunoreactivity was discarded. The pellet was solubilized by boiling for 2 min in electrophoresis sample buffer (0.25 M Tris-HCl, pH 8.6, 1.92 M glycine, 0.5% SDS, 2% mercaptoethanol) and protein concentration was determined by the BCA assay [56]. Proteins were resolved by polyacrylamide gel electrophoresis, transferred to nitrocellulose and the membranes blocked with 20% fetal bovine serum (FBS) in TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.01% Tween 20). The immunoblot was probed with anti-C_7 rabbit serum (generously provided by D. Selkoe) made to a synthetic peptide corresponding to the C-terminal region of APP [13] amino acids 732–751 according to the numbering of Ponte et al. [9]. Immunoreactive bands were detected with an alkaline phosphatase-conjugated goat anti-rabbit IgG (Promega protoBlot-Western system, Madison, WI) using BCIP/NBT substrates or with the PhotoBlot system (Gibco/BRL, Gaithersburg, MD). As a control for antibody specificity, immunoblots were incubated with anti-C_7 in the presence of 1 μg of the synthetic peptide (a kind gift from T. Kunishita and S. Younkin, CWRU).

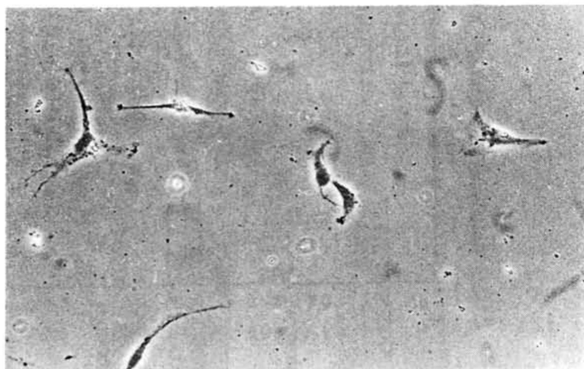
3. RESULTS

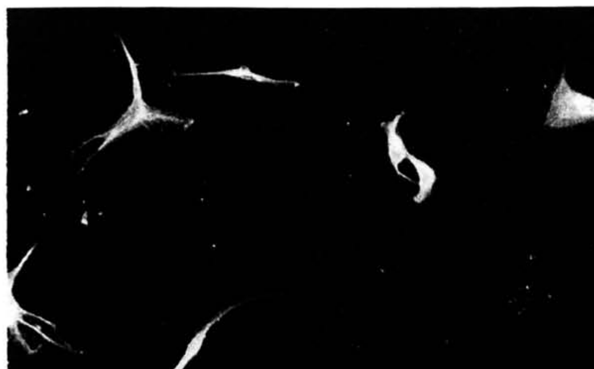
3.1. *Characterization of the primary cell cultures*

Astrocytes. Astroglial cells isolated from neonatal rat cerebral cortex (Fig. 1A) extended long processes during the first 2 DIV [48] and were GFAP-positive (+). These processes flattened thereafter and by 4 DIV, the cells had a fibroblast-like appearance and proliferated actively as expected of type 1 astrocytes. At 4 and 21 DIV, over 95% of the cells were GFAP(+).

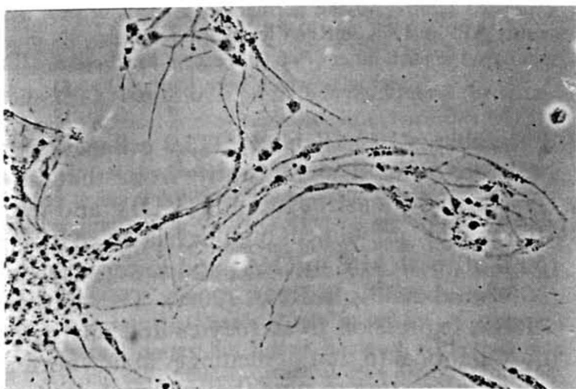
Neurons. Within 2 DIV, cortical neurons established long neuritic processes and over 95% of the cells were immunostained with an antibody to neurofilaments (Fig. 1B). Some of the other cells were GFAP(+) astro-

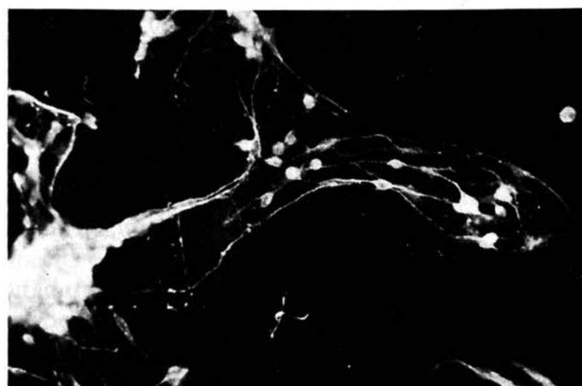
A. Astrocytes 2DIV





B. Neurons 2DIV





C. Microglia 11DIV

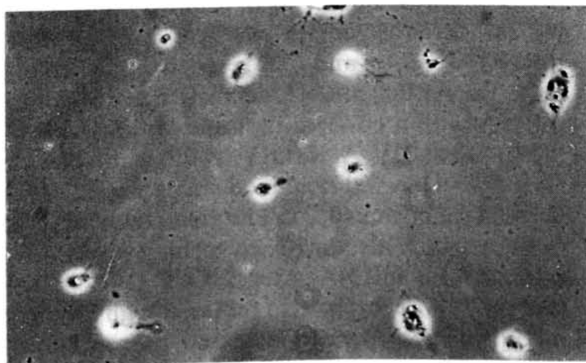


Fig. 1. Primary cultures of cortical astrocytes, neurons and microglia. (A) Astrocytes 4 DIV and (B) neurons 2 DIV were immunostained with an antiserum to GFAP and monoclonal antibody to neurofilaments, respectively. (C) Microglia cultures 11 DIV were incubated with AcLDL-DiI for 4 h. Immunoreactivity was detected in 95% of the cells in astrocyte and neuron cultures while 98% of the cells in microglial cultures ingested AcLDL-DiI.

glia (results not shown), while a few had the appearance of oligodendroglia [48].

Microglia. Over 98% of the cells isolated from cortical cultures at 10–30 DIV had the ability to phagocytize AcLDL-DiI (Fig. 1C). Most cells had the amoeboid appearance described for microglial cells upon initial isolation and, as expected of microglial cells, differentiated into process-bearing cells after a few days in cul-

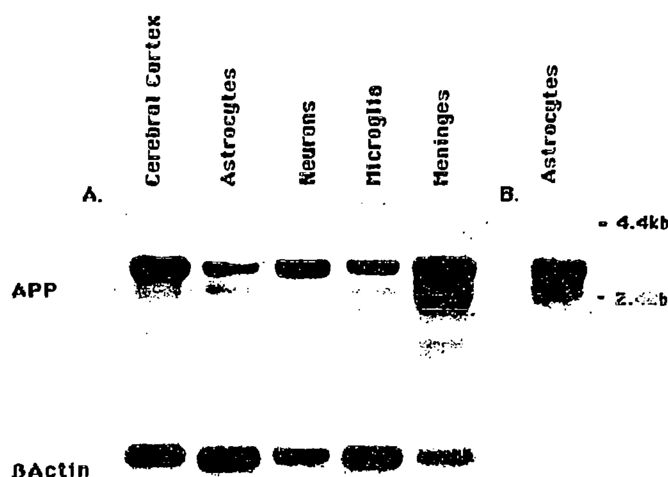


Fig. 2. Northern blot analysis of newborn rat cerebral cortex, meninges, and primary cultures of astrocytes, neurons and microglia. (A) Total cellular RNA (20 μg/lane) was probed for APP and β-actin mRNA content. (B) Astrocyte total cellular RNA (30 μg) was probed with a KPI-encoding cDNA. Size markers in kb (Pharmacia RNA ladder) are indicated on the right.

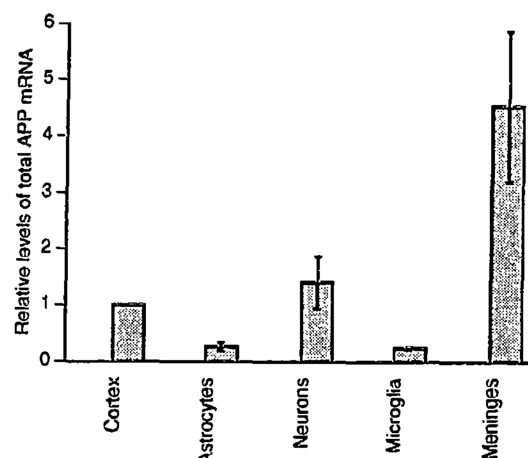


Fig. 3. Quantitative analysis of total APP mRNA by slot blot analysis in newborn rat cerebral cortex, meninges, primary cultures of astrocytes, neurons and microglia. Total APP mRNA levels from astrocytes (4 DIV), microglia (11 DIV), neurons (4 DIV) and meninges were expressed relative to the normalized level of newborn rat cerebral cortex APP mRNA. mRNA levels were significantly different when compared to each other ($P<0.003$) except for those of astrocytes and microglia, which were similar ($P>0.5$).

ture [44]. The other 2% of cells resembled oligodendrocytes [48] or were GFAP(+) (results not shown).

3.2. *Total APP mRNA is most abundant in meninges and in cortical neuronal cultures*

Total APP mRNA was detected as a 3.2–3.4 kb band in cortex, meninges, and cortical microglia, astrocytes and neurons (Fig. 2A). An additional mRNA of 2.7 kb which contains the KPI sequence (Fig. 2B) was relatively abundant in glial cells and highly expressed in meninges. The difference in size with the 3.2 kb mRNA excludes the possibility that this mRNA is one of the major known forms of APP mRNA. In addition, an APP mRNA of 2 kb was detected in meninges. Relative to cortical levels, APP mRNA quantitated by slot blot analysis, was most abundant in the meninges (4.5-fold), and neurons of 1–4 DIV (1.4-fold). In contrast, astrocytes at 4 DIV (0.26-fold) and microglial cells from 10 DIV cortical cultures (0.24-fold) had lower levels of APP mRNA (Fig. 3).

3.3. *Differential expression of APP transcripts is cell specific and influenced by cell culture conditions*

The relative abundance of APP_{695}, APP_{751} and APP_{770} mRNA assessed by RPA revealed alternative splicing patterns specific to neurons and glial cells (Fig. 4A). Newborn rat cerebral cortex (0 DIV) which contains mostly neurons [57] and neuronal cultures expressed APP_{695} mRNA predominantly whereas meninges, astrocytes and microglia contained mostly KPI-containing APP mRNAs. Quantitation of the RPA results showed that cortical neurons (2 DIV) expressed APP_{695} mRNA as 93% of total APP mRNA as well as low levels of APP_{751} mRNA (7%) while APP_{770} mRNA was barely detectable (<1%) (Table I). Glial cell APP mRNA expression differed significantly from that of neurons ($P<0.02$). Both astrocytes at 2–4 DIV and microglia at 10–30 DIV contained high levels of APP_{751} mRNA (50% of total APP mRNA) and lower levels of APP_{695} (30%) and APP_{770} mRNA (20%). The pattern of APP mRNA expression in astrocytes and microglia was almost identical to that of meninges in newborn rat brain. It was interesting to note that within 4 days of culture, the pattern of APP mRNA expression changed in cortical cultures from predominant APP_{695} mRNA to higher KPI-containing mRNA expression (Fig. 4A and Table I). Quantitative analysis of RPAs revealed that APP_{695} mRNA represented ~78% of total APP mRNA in neonatal rat cerebral cortex whereas APP_{751} and APP_{770} mRNA were 15 and 7% respectively (Table I). By 4 DIV, the APP_{695} mRNA decreased to 36% of total APP mRNA with a concomitant increase in APP_{751} mRNA (51%) ($P<0.01$). At 6 DIV, APP_{695} mRNA decreased further to 11% and remained stable until 21 DIV. Four-day-old rat cortex (0 DIV) retained high levels of APP_{695} mRNA which also switched to high levels of KPI-containing APP mRNA after 3 DIV (Fig. 4B). Furthermore, adult rat brain also retained high levels of APP_{695} mRNA. These results indicated that either a splicing switch occurs in the cerebral cortex with time in culture or the cell population of cultured cerebral cortex differs significantly from that of developing cerebral cortex.

Differential APP mRNA expression changed significantly in astrocytes maintained in culture for 20 days or longer (Table I). The level of APP_{695} mRNA decreased from 36 to 6% with a reciprocal increase in APP_{770} mRNA from 14% to 49% ($P<0.005$) whereas the relative amount of APP_{751} mRNA did not change with time

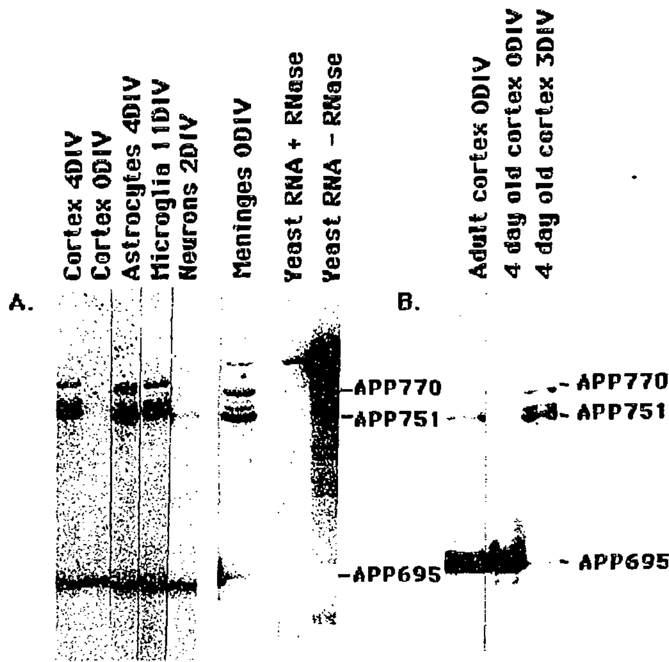


Fig. 4. Differential APP mRNA expression in rat cerebral cortex and primary cultures of cortical astrocytes, microglia and neurons. (A) The protected APP fragments resulting from ribonuclease protection assays (5–10 μg total RNA) were separated on denaturant 5% polyacrylamide gels. High levels of APP_{695} mRNA were detected exclusively in neurons and cerebral cortex (0 DIV) whereas high levels of KPI-containing APP mRNA were observed in meninges, microglia and astrocytes as well as in whole cortical cultures of 4 DIV. Intact probe (Yeast RNA − RNase) is almost all digested with RNase treatment (Yeast RNA + RNase). High levels of APP_{695} mRNA in adult and 4-day-old rat cerebral cortex but higher levels of KPI-containing mRNAs in 4-day-old rat cerebral cortex cultured 3 days. Note that different amounts of RNA and exposure times were used to facilitate detection.

in culture. Over 95% of the cells in astrocytic cultures remained GFAP(+) at 21 DIV, eliminating the possibility that the switch in APP gene expression is the result of a changing cell population with time in culture. A similar shift in APP mRNA expression was also observed in neurons between 3 and 4 DIV (Table I). However, it was not established whether the switch is an intrinsic feature of neuronal cells maintained in culture or whether it is due to a change in cell population.

3.4. *The type of cellular APP represents differential APP mRNA expression in each tissue and cell type but the relative amount of protein does not parallel APP mRNA levels*

Immunoblots of cellular proteins with antibodies to the C-terminal fragment of APP revealed that the pattern of APP proteins paralled that of APP mRNA (Fig. 5A). Competition expeirments eliminated all immunoreactive bands except two (Fig. 5B). Rat neonatal cortex displayed an intense APP band of approximately 110 kDa which is likely to be APP_{695}, and a less intense slightly higher mol.wt. band similar to a previous report [58]. Two additional higher mol.wt. bands were detected in the meninges and are probably the KPI-containing forms of APP. Neuronal APP proteins were similar to those of cortex, with a major APP band migrating at 110 kDa. Astrocytes appeared to have multiple APP species ranging from a scarcely expressed species of 110 kDa to 4 more abundant forms above 110 kDa. Surprisingly, the steady-state level of APP was lower in meninges although these contained the highest level of APP mRNA, and was barely detectable in microglial cells which contain as much APP mRNA as astrocytes.

Table I

Differential APP mRNA expression in newborn rat cerebral cortex, meninges and primary cultures of cerebral cortex, astrocytes, neurons and microglia

Sample	DIV	*n*	APP_{695}	APP_{751}	APP_{770}
Cortex	0	4	0.78 ± 0.15	0.15 ± 0.10	0.07± 0.06
	2	3	0.81 ± 0.02	0.16 ± 0.01	0.03 ±0.01
	4	3	0.36 ± 0.09	0.51 ± 0.08	0.13 ± 0.06
	6	2	0.11, 0.18	0.60, 0.66	0.29, 0.16
	21	3	0.11 ± 0.00	0.78 ± 0.04	0.11 ± 0.04
Meninges	0	7	0.30 ± 0.07	0.49 ± 0.07	0.22 ± 0.04
Astrocyte	2	1	0.36	0.50	0.14
	4	6	0.29 ± 0.09	0.50 ± 0.10	0.21 ± 0.11
	>21	5	0.06 ± 0.04	0.45 ± 0.03	0.49 ± 0.03
Neuron	2	3	0.93 ± 0.07	0.07 ± 0.06	0.01 ± 0.01
	3	2	0.93, 0.83	0.06, 0.12	ND
	4	4	0.63 ± 0.07	0.28 ± 0.06	0.08 ± 0.07
Microglia	10–30	3	0.24 ± 0.16	0.61 ± 0.15	0.15 ± 0.09

The proportion of each APP mRNA in specific cells and tissues was determined by densitometric scanning of the RPAs. Values for each APP mRNA transcript are expressed as a fraction of the normalized sum of all three APP mRNAs. Each value represents the mean of *n* samples ± standard deviation. ND = not determined.

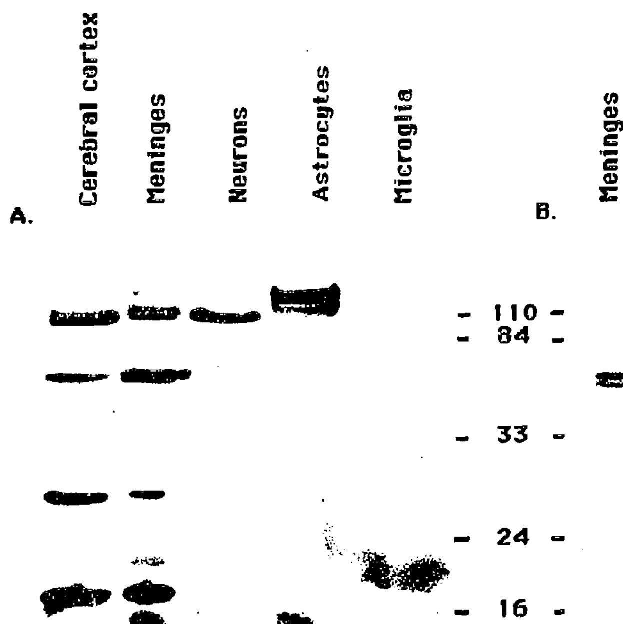


Fig. 5. Western blot analysis of cellular APP proteins from newborn rat cerebral cortex, meninges and primary cortical cultures of astrocytes, neurons and microglia. (A) Cellular proteins (20 μg/lane) were resolved on 10% polyacrylamide gels. Immunoblots with anti-C_7 antibodies detected full length APP species in newborn rat brain cortex (0 DIV), meninges (0 DIV), astrocytes (4 DIV) and neurons (2 DIV) but not in microglial cells (10 DIV). In addition, two smaller immunoreactive APP proteins of 16 and 26 kDa were detected in cerebral cortex and meninges. Except for the faint presence of the 26 kDa band in neurons, these were not detected in any of the primary cell cultures. (B) Immunoreactivity of anti-C_7 antibodies with all these proteins was specifically competed with C-peptide with the exception of a 67 kDa band.

3.5. *Low mol.wt. C-terminal fragments are not detected in the primary cell cultures*

In addition to the full length APP forms, the anti-C_7 antibody specifically detected 2 lower mol.wt. species of 26 kDa and 16 kDa in the cortex and meninges. The 26 kDa protein was weakly detected in neurons but not in glial cultures whereas the 16 kDa protein was not observed in any of the primary cell cultures (Fig. 5). These immunoreactive proteins are likely to be C-terminal fragments of APP.

4. DISCUSSION

APP gene expression was investigated in newborn rat cerebral cortex, meninges and primary cultures of cortical neurons, astrocytes and microglia to gain insight into the possible contribution of individual CNS cells in βAP deposition. The highest level of APP mRNA was found in the meninges. High levels of APP mRNA were detected in neurons in agreement with most in situ hybridization studies [17–24]. Microglia and astrocytes contained an equivalent amount of APP mRNA which was lower than that of neurons. The higher steady-state level of APP mRNA in neurons compared to glial cells indicates cell-specific transcriptional regulation of APP gene expression which may be relevant to the functional role of APP in these different cell types.

The high levels of APP mRNA in meninges and neurons may be relevant to the deposition of amyloid in meningeal and cerebral cortical vessels as well as within the parenchyma of the cerebral cortex in AD. In hereditary cerebral hemorrhage with amyloidosis of Dutch type, a disease characterized by extensive βAP deposits in leptomeningeal and cortical vessel walls, a point mutation in the βAP sequence has been identified and suggested to be the cause of amyloid deposition in this disease [59]. Another mutation in the APP gene has been identified in familial AD [60–62]. This mutation disrupts a stem loop structure of APP mRNA that may impair translational regulation leading to over-expression of the APP and abnormal levels of βAP [63] as observed in Down's syndrome patients. Therefore, high levels of APP mRNA in neurons and meninges coupled with abnormally high translation of the mutant APP mRNA could lead to substantial levels of βAP deposition if over-expression is indeed a primary cause of abnormal deposition of βAP.

We observed that not only the level of total APP mRNA but also the ratios of the various APP mRNA species were different in neurons and glial cells indicating cell-specific post-transcriptional regulation at the splicing level. In newborn rats, neurons expressed high levels of APP_{695} mRNA whereas microglia and astrocytes expressed higher levels of KPI-containing APP mRNA. This pattern of APP mRNA expression was comparable to that reported for the mouse [39], although we detected significant levels of APP_{695} mRNA in rat astrocytes at 2–4 DIV and microglia cultures. It has previously been reported that type I astrocyte cultures do not express KPI-containing forms of APP [40]. In our culture system, the RNase protection assay and Northern blots with a probe containing only the KPI-encoding APP cDNA sequence, clearly revealed a high level of KPI-containing APP mRNA in astrocyte primary cultures.

The change in differential APP mRNA expression in astrocytes with increasing time in culture is indicative of a switch in alternative APP mRNA splicing. Whether this switch in splicing occurs in aging astrocytes in vivo or is the result of the culture conditions remains to be determined. Higher proportions of APP_{751} mRNA and APP_{770} mRNA have been detected in AD cases compared with normal controls [37,64] and in rats with spatial memory deficits [65] not unlike that observed in our cultures. The switch in splicing was also observed in cerebral cortical cells maintained for some time in culture. However, intact cerebral cortex of adult rats retained high expression of APP_{695} mRNA. It is possible that the cell population that evolves in cultured cerebral cortex differs from that of developing cerebral cortex.

There were also remarkable discrepancies in some cell types between the amount of APP mRNA and that

of APP isoforms. Microglia, which displayed APP mRNA levels comparable to that of astrocytes, contained barely detectable amounts of APP which were instead relatively abundant in astrocytes. Meninges, which exhibited the highest level of total APP mRNA, contained less APP than neurons and astrocytes. These results are consistent with either negative translational regulation of APP gene expression or enhanced turnover of APP in microglial cells and meninges, whereas increased translational efficiency or lower turnover of APPs may explain the relatively abundant APPs in astrocytes. A high turnover rate of APPs would support the postulate that microglia are producers as well as processors of βAP peptide [25]. Further studies, such as pulse chase experiments, will determine which of these mechanisms regulates the steady-state levels of APP proteins in these cells.

The low levels or absence of immunoreactive peptides of 26 and 16 kDa in neuronal and astroglial cultures is intriguing. These proteins are most likely C-terminal fragments resulting from proteolytic processing of APPs. Similar C-terminal fragments of APP have been detected in rat brain membranes [66], APP-transfected 293 cells [67] and PC12 cells [68]. The absence of these fragments in primary culture systems raises the possibility that post-translational processing of APP or turnover of APP C-terminal fragments changes in culture conditions.

In addition to the normally observed 3.2 kb mRNA, meninges expressed APP mRNAs of 2.7 kb and 2.0 kb. Astrocytes and microglial cells also contained relatively abundant levels of the 2.7 kb APP mRNA species. An APP mRNA species of similar size was detected previously in rat tissue [69]. We show that the 2.7 kb APP mRNA contains the KPI domain and preliminary results indicate that the βAP region is also present. Further studies are underway to determine the exact nature of this form of APP mRNA. The 2 kb band in meninges is probably APP_{563} mRNA [12].

In conclusion, our results suggest that regulation of APP gene expression is highly complex and under tight control in specific cell types of the CNS. Although the APP gene expression in short-term cultures reflects the in vivo situation, long-term cell cultures appear to change the pattern of alternatively spliced APP mRNAs and possibly affect post-translational processing of APP as well. Therefore, it is reasonable to believe that disruptions of the CNS environment will influence the regulation of APP gene expression in neurons, microglia and astrocytes and that any of these cells could be responsible for βAP deposition under appropriate conditions.

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