Expression of Neuronal mRNAs in Alzheimer Type Degeneration of the Nervous System

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ABSTRACT: There is extensive evidence for decrements of gene expression in AD, at several levels in the process. There is also evidence for increments of expression of some genes. Message for the amyloid precursor protein (APP), for example, is elevated in surviving neurons of certain subcortical populations in AD. We evaluated expression of message for APP as well as for certain neuronal and glial cytoskeletal proteins in the cortex of six cases of AD. Neuronal mRNAs, including that for APP, were significantly decreased when compared with control cortex, whereas the glial mRNA was increased. We have projected studies to determine the evolution of these mRNA decrements in Alzheimer-type degeneration. The rationale for these studies and preliminary findings are discussed.

RÉSUMÉ: Expression d'ARN messagers neuronaux dans la dégénérescence de type Alzheimer du système nerveux Il existe de données nombreuses indiquant qu'il y a une diminution de l'expression génique dans la maladie d'Alzheimer, et ce à plusieurs niveaux dans le processus. Il existe également des données indiquant une augmentation de l'expression de certains gènes. Le messager codant pour la protéine précurseur de la substance amyloïde (APP), par exemple, est augmenté dans les neurones restants de certaines populations souscorticales dans la maladie d'Alzheimer. Nous avons évalué l'expression du messager de l'APP ainsi que de certaines protéines du cytosquelette neuronal et glial dans le cortex de six cas de l'APP, étaient significativement diminués par rapport aux cortex témoins, alors que l'ARN messager glial était augmenté. Nous projetons d'étudier l'évolution de ces diminutions d'ARN messagers dans la dégénérescence de type Alzheimer. Nous discutons de la justification de ces études ainsi que des résultats préliminaires.

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Neuronal gene expression is extensively altered in Alzheimer's disease (AD). Information from many sources indicates that changes occur at several levels, from gene transcription[1] to post-translational processing of proteins.[2]

Decrements in Gene Expression

Numerous reports have provided evidence that gene expression is decreased in AD, but there are discrepancies among these studies in certain details. McLachlan and colleagues have conducted a series of studies indicating changes in chromatin of both neurons and glia.[1,3,4] The changes support the concept that DNA is less accessible to transcription in AD brain than in controls. Nucleolar size is diminished in neurons containing neurofibrillary tangles (NFT) when compared to neighboring non tangle-bearing neurons in the temporal cortex of AD;[5] and in neurons of AD cortex when compared to control cortex.[6] Similarly, total RNA is reduced in neurons of AD subiculum compared to controls; and neurons with NFT show the most marked reduction.[7] Despite reduction of total RNA in individual cells,[6,7] the total RNA isolated per unit weight of AD cortex was normal in some studies,[8,9] whereas others have found a decrement in AD.[10]

Decrements of RNA within degenerating cells raise the possibility that RNA degradation is increased. Evidence for increased ribonuclease activity in AD was reported by Sajdol-Sulkowsha and Marotta,[11] but other laboratories have found no differences in ribonuclease activity between AD and control.[8,12]

Most cellular RNA is ribosomal, and assessment of messenger RNA requires other approaches such as isolation of poly A RNA, in vitro translation, and molecular hybridization for specific mRNAs. Guillemette et al[8] found a reduction of poly A RNA, both when expressed as µg/g of tissue or when expressed as a percentage of total RNA; these results are consistent with those of Sajdel-Sulkowska and Marotta[11] and of Taylor et al,[13] but differ from some other reports.[9,14] In vitro translation has demonstrated quantitative differences between AD and control.[9,10] Sajdel-Sulkowska and Marotta[10] found decrements of poly A RNA and translational activity of mRNA from AD

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brain. However, Langstrom and colleagues[9] found no loss of poly A RNA, or of translational activity of poly A RNA in AD cortex. Instead, they found a reduction of translation from AD polysomes, due to some factor other than their poly A component.

Increments in Gene Expression

Deprivation of nerve growth factor is followed by an increment in expression of some genes in cultured sympathetic neurons; and the death of these cells can be prevented by inhibitors of protein synthesis.[15] Deprivation of trophic substances, as hypothesized for AD,[16,17] might therefore lead to increments in gene expression which are potentially lethal to the cell. This observation emphasizes the importance of searching for mRNAs which are overexpressed in AD, as described by Dr. Patrick May in this symposium.

Another line of evidence for increments in expression of specific genes in AD is related to studies of Down's syndrome. Even in their third decade, individuals with trisomy 21 develop Alzheimer type degeneration,[18] and the changes are usually conspicuous after the age of 35 years.[19] Since an extra copy of chromosome 21 predisposes to Alzheimer type degeneration, it seems plausible that overexpression of one or more genes on that chromosome may be pathogenetic in AD itself.

This hypothesis attracted further interest when chromosome 21 was linked to the cerebral amyloid which accumulates in AD. A major component of AD amyloid is a 43 amino acid polypeptide, which is the cleavage product of a much larger protein, termed the Alzheimer amyloid precursor protein (APP). The form of the APP first described has a primary structure of 695 amino acids (APP_{695}).[20] Longer forms of the APP (APP_{751}, APP_{770}), containing a protease inhibitor domain, are inferred from the existence of alternate forms of the message.[21-23] The mRNAs for APP all derive from a single gene located on chromosome 21[24,25] and are abundantly though not exclusively expressed by neurons.[22,24,26-29] These findings suggested that overexpression of the APP gene might play a primary pathogenetic role in AD itself.

The concept that individuals with AD had an extra copy of the APP gene was supported in one study,[30] but refuted in a series of subsequent reports.[31-33] Whether increased transcription of this gene or enhanced stability of the message plays a pathogenic role in AD is still an open question. Recent studies have provided evidence for elevated expression of the APP mRNA in the nucleus basalis of Meynert[34] and locus ceruleus.[34,35] Palmert and colleagues found this increase referable to the APP_{695} form of the message. APP mRNA may also be overexpressed in parts of the hippocampal formation, but there are discrepancies among the available studies.[29,35,36] The potential influence of APP on neuronal survival has been the subject of several reports.[37-39]

Decrements of mRNAs for APP and the Neuronal Cytoskeleton in AD Cortex

We have studied expression of the APP mRNA in AD-afflicted cortex, and compared this with expression of mRNAs for proteins of the neuronal and glial cytoskeleton.[40] The neurofilament light subunit (NF-L) is stoichiometrically the most abundant of the neurofilament proteins; its mRNA is expressed only by neurons. Tubulin is expressed in all cell types, but the probe for α1-tubulin used in this study hybridized predominantly with neuronal mRNA when tested in situ in our laboratory. In addition to neuronal mRNAs, we evaluated the mRNA for glial fibrillary acidic protein (GFAP). Since this message is expressed only by glia, it is useful for comparison with neuronal mRNAs.

We compared mRNA expression in parietal cortex obtained postmortem from 6 AD subjects (64-81, mean 74.2 years of age) with that obtained from 6 controls (55-82, mean 72.5 years). The AD cases were selected for typical, extensive Alzheimer type pathologic changes, with a mean NFT count of > 16/mm^2 and amyloid plaque (AP) count of > 24/mm^2 in parietal cortex.

The method employed was that of Northern analysis. Total RNA was extracted from parietal cortex which had been frozen at –70° the day of autopsy. Our yields of total RNA were 514 ± 17 μg/g tissue from controls and 394 ± 45 μg/g from AD cases (mean ± SEM). Two of the AD cases had total RNA yields in the control range, but the data showed a significant difference between the group means.

The cDNA for APP which we used as probe was derived from a library of postmortem human, Alzheimer-afflicted brain cDNAs, and was provided by Dane Liston.[40] The probe, as prepared for this study, would be expected to hybridize to all forms of the APP message without distinguishing among them. The probe for NF-L was derived from a human embryonic brain genomic DNA library and was the gift of Jean-Pierre Julien.[41] Probes for α1-tubulin and GFAP message were from mouse brain cDNA libraries and were gifts of Nicholas Cowan.[42,43]

From each AD and each control case, an equal amount of total RNA was loaded onto a designated lane of an agarose-formaldehyde gel and the RNAs electrophoretically separated by size (Figure 1).

Following transfer of the RNAs to a nylon membrane, hybridization to each of the selected probes and autoradiography gave results as illustrated in Figure 2. Because of variable degradation in controls and AD cases, we used the total hybridization signal intensity, including the band and the smear of degradation products below it, when quantitating the mRNA

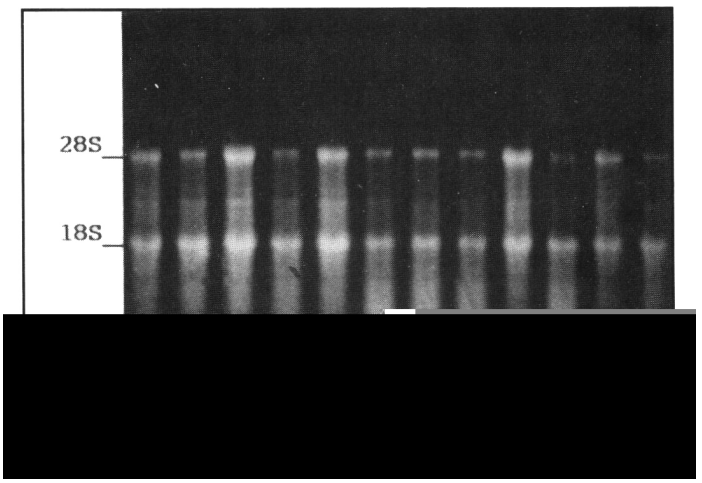

Figure 1 — Agarose/Formaldehyde RNA Gel. Each odd-numbered lane contains 15 μg total RNA from a control case, each even-numbered lane contains 15 μg total RNA from an AD case. The gel has been stained with ethidium bromide and visualized under UV light. The 28S bands are clearly identifiable for each case, with some variability.

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for each case by densitometry. This densitometry value for each case indicates the relative amount of the mRNA of interest (Table 1).

A message integrity index (MI, equal to the signal intensity of the band divided by the total signal intensity) was also calculated to determine the extent of degradation of each message individually. An MI value of 1.0 indicates intact message, whereas progressively smaller values indicate increasing degradation. Using this value, we found that tubulin message was less degraded in AD cases than in controls, while the other 3 messages were more degraded in AD. None of the differences in degradation was statistically significant, however.

This study revealed significant losses of the three neuronal mRNAs in AD cortex (Table 1). The APP mRNA was reduced to 52%, NF-L to 36%, and tubulin to 47% of the control value; whereas GFAP mRNA in AD cortex was increased to 133% of control. The decrements in neuronal mRNAs were greater than expected from neuronal loss alone.[40,44] The decline in mRNA for APP, of course, does not preclude the possibility that this message is elevated in certain neuronal populations or in earlier stages of Alzheimer type degeneration. The findings support the concept of impaired neuronal gene transcription, and indicate that, at least in late stages of the process, the APP gene is affected.

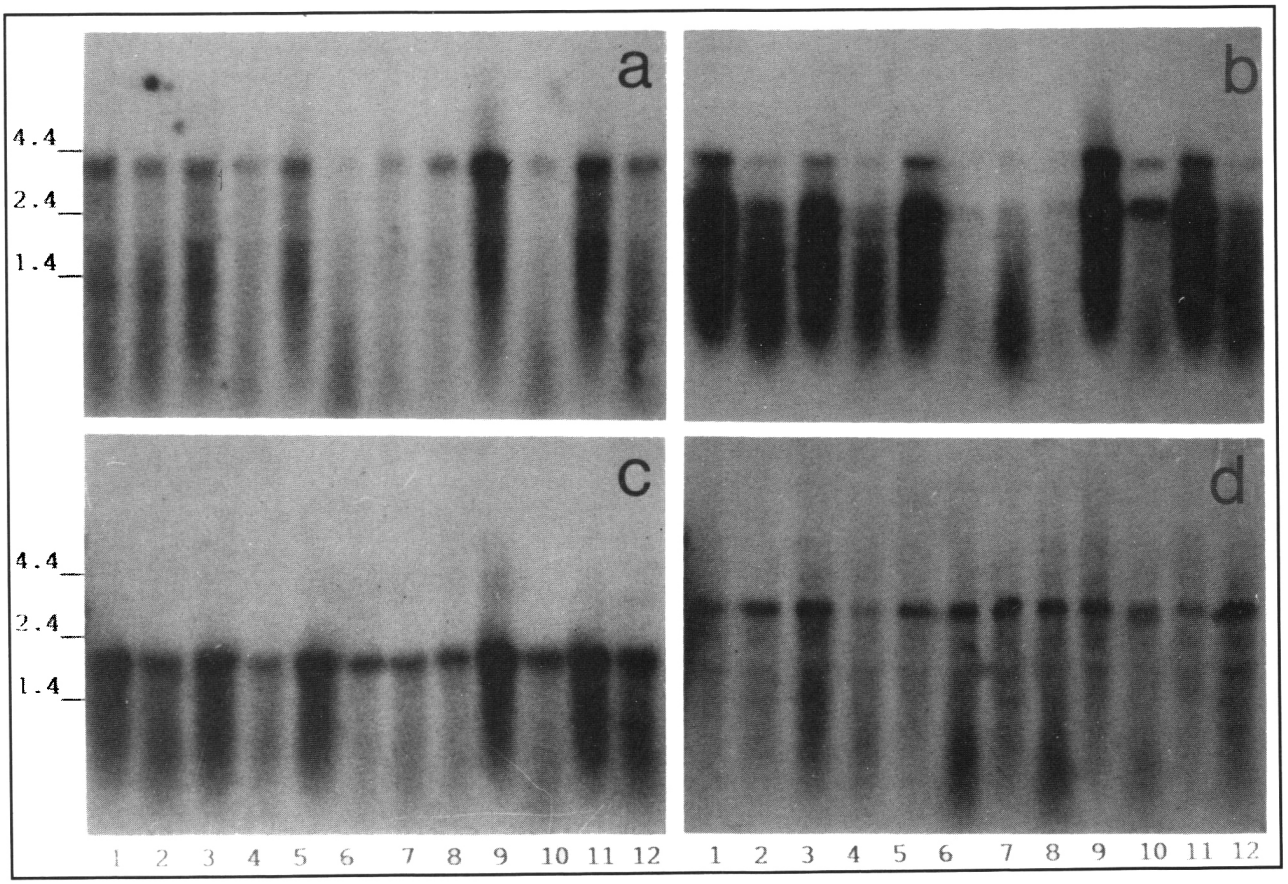

*Figure 2 — Autoradiograms of Northern transfers. The transfers were hybridized with **a)** APP, **b)** NF-L, **c)** α-1 tubulin, and **d)** GFAP probes. In each transfer the odd-numbered lanes represent controls, the even-numbered lanes represent AD cases.*

Table 1: Northern Analysis Densitometry

	APP	NF-L	α-1 Tubulin	GFAP
Control				
B	6.73 ± 1.04	1.26 ± 0.56	7.59 ± 1.09	1.93 ± 0.29
T	29.8 ± 3.48	54.3 ± 8.04	22.1 ± 1.61	7.3 ± 2.57
MI	0.23	0.02	0.34	0.42
Alzheimer				
B	2.47 ± 0.56*	0.24 ± 0.07*	3.84 ± 0.64*	1.98 ± 0.27
T	15.4 ± 2.82*	19.5 ± 5.65*	10.4 ± 2.64*	9.7 ± 1.91
MI	0.16	0.02	0.46	0.24

Densitometry of band (B) and total (T) signals are in arbitrary units. Values represent mean ± SEM. MI = message integrity index (see text). APP = amyloid precursor protein, NF-L = neurofilament light subunit, GFAP = glial fibrillary acidic protein.

*P < 0.05 on Mann-Whitney U test.

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Our data are consistent with those of McLachlan and colleagues[45] for the NF-L mRNA. Langstrom et al[9] found no decrement of mRNA or of translational activity for the heavy neurofilament subunit (NF-H).

Two other studies have identified decrements of APP mRNA in AD cortex. Goedert[27] found a reduction of mRNA for APP in AD frontal cortex, but interpreted this as due to loss of neurons. Using oligonucleotide probes specific for APP_{695} and for an insert common to longer forms of the message, Johnson and colleagues[46] found a 65% decrease in APP_{695} but no significant change in APP_{751} in AD cortex. They suggested that a relative increase in APP_{751}, a form of the protein containing a protease inhibitor domain, might be pathogenetically important in AD. Tanaka et al[47] found no significant change in APP_{695} or APP_{751} mRNA, but a significant increase in APP_{770} in AD. The differences between our findings and those of Tanaka et al are puzzling, but may be related to different choice of controls. Each of their four controls had some form of neuropathologic change, whereas we chose normal control brains.

mRNA Decrements and the Process of Alzheimer Type Degeneration

The foregoing evidence has persuaded us that there are decrements in expression of many neuronal mRNAs in AD, at least in the common senile form of the disorder. We could better understand the pathogenesis of AD if we knew whether these decrements appear only with severe stages of AT degeneration, or whether they are present when the histologic changes are less extensive. We will approach this question in two ways.

In the first, we will select subjects of AD and non-demented cases matched for severity of degenerative change. Among elderly non-demented subjects, autopsy reveals a variable extent of amyloid-related deposits in neuropil and of neuritic plaques.[48,49] In subjects with AD, there is also considerable variation.[50,51] Thirty per cent of the cases have many plaques but no tangles in the neocortex.[52] These case-to-case variations may reflect the temporal progression of AT degeneration. It should be noted, however, that individual cases of AD assessed first at biopsy and 3 to 7 years later at autopsy showed no consistent increase in tangles or plaques, although neuronal loss continued in the intervening years.[53] An alternate interpretation of differences among cases in the extent of Alzheimer type degeneration is that they represent a spectrum of endpoints, each unique to the case in which it is observed. AD cases lacking neocortical tangles, for example, may be resistant to their formation in this area, regardless of disease duration.

By correlating mRNA expression with plaque formation and with NFT formation, we will clarify the level of severity at which the decrements appear. The general sequence of events can probably be inferred from these data. Our findings to date indicate that decrements of mRNAs for NF-L and APP correlate directly with the extent of NFT formation in AD cortex. *Increments* of mRNA for GFAP also correlate directly with tangle counts (unpublished). We would predict from this that the mRNA decrements will not be detectable in cortex of AD cases without cortical NFT.[52]

A second approach to the evolution of mRNA decrements in Alzheimer type degeneration involves the study of subjects with trisomy 21 at all ages from fetal through late adult life. We designed this study with the concept of Down's syndrome (DS) as a paradigm for AD. We have begun a study of postmortem mRNA from cortex of DS cases at ages from fetal through late adult life. We studied APP mRNA and NF-L mRNA levels, comparing them in each case with the extent of Alzheimer type degeneration. Since the APP mRNA is derived from chromosome 21 and the NF-L mRNA from chromosome 8, we expected the younger DS subjects (with 1.5 times the normal number of copies for chromosome 21 genes) to produce significantly more APP mRNA but about the same level of NF-L mRNA compared to controls. Our original intent was to define, by this alternate approach, the stage at which mRNA decrements would appear in the process of AT degeneration.

As expected, we find elevated levels of APP mRNA, and normal levels of NF-L mRNA, in the cortex of fetal and young adult DS subjects. In other respects, however, our results to date have come as a surprise. They suggest that DS subjects are remarkably resistant to decrements of neuronal mRNA, even in the presence of extensive Alzheimer type degeneration.[54] These preliminary results suggest that there are significant differences between Alzheimer type degeneration in DS and in the senile form of AD at the level of neuronal gene transcription or RNA processing. One possibility is that the difference is a function of age, the DS subjects with Alzheimer type degeneration being 20 to 40 years younger than the subjects with comparable severity of degeneration in AD.

Comment

The central role of mRNA metabolism in structure and function of the cell mandates an effort to understand this aspect of Alzheimer type degeneration. The studies will necessarily include human postmortem tissue, because of the limitations of available animal models.

Discrepancies among reports of gene expression in AD may be related to several factors. AD occurs in an aging population; and decrements of gene expression occur in aging brain independently of AD.[10] AD itself is not a homogeneous disorder. Thus, real in vivo differences in populations studied must be taken into account.

Most troublesome, however, is the persistent problem of variable degradation of RNA, both among controls and among subjects with Alzheimer type degeneration. Circumstances between death and freezing of tissue may play a role, although we have recovered RNA of excellent quality from tissue frozen more than 24 hours after death, and we find no significant correlation between RNA quality and postmortem intervals under 30 hours. We believe the degradation is related chiefly to factors in the immediate antemortem period. The many variables affecting humans in this interval are effectively controlled in experimental studies of animal tissue, with the result that mRNA obtained in the latter type of study is more consistently well preserved. Increasing the sample size can compensate for this, but it would be helpful to identify the factors responsible for degradation. Selection and control for these factors would improve precision of the studies and permit more efficient choice of case material.

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