THE EFFECT OF AMYLOID ASSOCIATED PROTEINS ON THE EXPRESSION OF GENES INVOLVED IN AMYLOID-BETA CLEARANCE BY ADULT HUMAN ASTROCYTES

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Submitted
ABSTRACT

Astrocytes appear to be important mediators in the clearance of amyloid beta1-42 (Aβ), the key component of senile plaques characteristic of Alzheimer’s disease (AD). Recently, we found amyloid associated proteins (AAPs) including α1-antichymotrypsin (ACT), apolipoprotein J and E (ApoJ and ApoE) and a mixture of serum amyloid P (SAP) and C1q (SAP-C1q) to modify Aβ uptake by human astrocytes. Here we investigated the effect of oligomeric (Aβ oligo) and fibrillar Aβ (Aβ fib), alone and in combination with AAPs (Aβ-AAPs), on the astrocytic expression of genes proposed to be involved in Aβ-uptake and degradation. Primary human astrocytes (isolated from non-demented control (n=4) and AD patient (n=4) brain specimens) were exposed to either Aβ oligo or Aβ fib, alone or with or without the above mentioned AAPs. Quantitative gene expression analysis of Aβ receptors (Scavenger receptor B1 (SCARB1), MARCO and LRP2) and Aβ degrading enzymes (neprilysin (NEP), insulin-degrading enzyme (IDE) and metalloproteinase 9 (MMP-9)) was performed by real time PCR. Basal expression of NEP, IDE and SCARB1 by the astrocytes was clearly detectable, whereas expression of MARCO, LRP2 and MMP-9 could only be detected upon pre-amplification. Aβ fib and Aβ oligo alone did not affect expression of NEP, IDE and SCARB, whereas several Aβ-AAPs, mainly apolipoproteins, up-regulated NEP and SCARB and down-regulated IDE in control astrocytes. No such modulating effects were seen in AD astrocytes. These data suggest that interaction of Aβ with apolipoproteins influences the expression of enzymes and receptors involved in Aβ clearance in astrocytes and that this astrocytic mechanism is defective in AD.
INTRODUCTION

Alzheimer’s disease (AD) is characterized by the accumulation and deposition of amyloid beta (Aβ) peptides within the brain, leading to the perturbation of synaptic function and neuronal loss. A failure of Aβ clearance in human brain seems to be the most likely cause in sporadic AD. Therefore, enhanced Aβ clearance induced by Aβ vaccine is currently under investigation in several clinical trials. The mechanisms underlying enhanced clearance however are not fully elucidated. Several pathways for Aβ clearance have been suggested: 1) Aβ clearance over the blood brain barrier 2) degradation by proteolytic enzymes 3) Aβ uptake and degradation by glial cells. The effect of even modest increases in Aβ levels in the brain accumulated over time is thought to be highly significant in the development of AD.

Astrocytes are located in close proximity to amyloid plaques. Aβ positive granules have been identified in astrocytes in the human brain and in previous in vitro studies, we have shown that primary human astrocytes in culture can bind and ingest Aβ. These data support the assumption that astrocytes play an important role in plaque maintenance and amyloid clearance.

Apart from Aβ several other proteins co-localize with amyloid plaques, the so called amyloid associated proteins (AAPs). AAPs that were found to co-localize with Aβ deposits before tau pathology and glial activation occurs were of special interest in this study. They may reflect a very early stage of AD pathology and consist of serum amyloid P (SAP), complement factor C1q (C1q), alpha1-antichymotrypsin (ACT), apolipoprotein J (ApoJ) and apolipoprotein E (ApoE). For example, Aβ in combination with SAP and C1q can inhibit Aβ uptake in microglia and it can elicit a stronger pro-inflammatory response in human microglia compared to Aβ alone. ACT is unique for Aβ containing plaques and it can enhance AD pathology. A recent genome-wide association studies repeatedly identified ApoJ, next to ApoE, as a genetic risk factor for AD. Furthermore, both ApoJ and ApoE have been proposed earlier to affect Aβ clearance, implicating a very important role of these AAPs in Aβ clearance. The mechanisms underlying these effects clearly need to be elucidated.

It is unknown how astrocytes internalize and degrade Aβ, however it seems that Aβ conformation is an important determinant. Scavenger receptors have been implicated in binding and uptake of several compounds including Aβ. Especially scavenger receptor class B member 1 (SCARB1) and the macrophage receptor with collagenous structure (MARCO; member of scavenger receptors class A) have, based on experimental animal studies, been proposed as important players in Aβ binding and uptake. The low density lipoprotein receptor related protein 2 (LRP2 or megalin) is involved in the endocytic uptake and degradation of Aβ bound to ApoJ. At the blood-brain-barrier and blood-CSF barrier, LRP2 plays a role in mediating both influx
and efflux of Aβ in the brain, when complexed to ApoJ 27, 28. All three receptors have been shown to be expressed on either rodent or human astrocytes 23, 29-32.

Next to possible Aβ receptors, astrocytes also express several Aβ degrading proteases. Several Aβ-degrading proteases have been identified by their ability to cleave Aβ, including neprilysin (NEP), insulin-degrading enzyme (IDE), endothelin-converting enzyme (ECE), angiotensin-converting enzyme (ACE), the plasminogen activators and matrix metalloproteinase (MMP) -9 and MMP-2 . Although all these proteases were shown to degrade Aβ in vitro, NEP and IDE probably are the predominant Aβ degrading enzymes in vivo 33-35, with profound expression in human astrocytes 36, 37. Of all Aβ degrading enzymes, only MMP-9 is capable of cleaving Aβ and expression in mouse astrocytes was shown to contribute to extracellular brain Aβ clearance 38, 39. Taken together, these studies indicate an important role of the above mentioned Aβ receptors and Aβ degrading proteases expressed by rodent astrocytes in Aβ clearance. However little is known about the expression of these receptors and enzymes by human astrocytes.

In this study we targeted the astrocytic expression of the proposed Aβ-receptors SCARB1, MARCO, LRP2 and the Aβ-degrading enzymes NEP, IDE and MMP-9. We aimed to 1) Investigate the expression of these genes in primary human astrocytes and if the expression differs between astrocytes isolated from brain specimens of non-demented control subjects and AD patients 2) Study the effect of Aβ oligo and Aβ fibr on expression of above mentioned genes 3) Study the possible additive effects of AAPs on the Aβ oligo and Aβ fibr induced expression of the genes. The ultimate goal of our investigation is to test the hypothesis that human astrocytes express mRNA of several Aβ receptors and Aβ degrading enzymes, and that the Aβ uptake and degradation capacity can be altered due to modulatory effects of Aβ and AAPs on gene expression.

**MATERIAL AND METHODS**

**Primary human astrocyte cultures**

Adult primary astrocytes were isolated from human brain specimens obtained at autopsy (through the Netherlands Brain Bank) 40 or obtained at surgery (focal cortical temporal resection for intractable complex partial seizures). Patients gave informed consent, and the use of tissue for experiments was approved by the local Medical Ethics Committee at the VUmc, in compliance with the Declaration of Helsinki. Astrocytes derived from AD brain tissue (n=4) that were clinically diagnosed with AD, and control brain tissue (n=4) from non-demented control subjects (Table 1), were characterized based on glial fibrillary acidic protein (GFAP) immunoreactivity and cultured as described before 9, 41.
**Aβ and AAPs preparations**

Preparation of 10 µM oligomeric and fibrillar Aβ1-42 (Bachem, Bubendorf, Switzerland) species (Aβ\textsubscript{oligo} and Aβ\textsubscript{fib}) were prepared and characterized essentially as described before \textsuperscript{8, 42, 43}. All Aβ samples for the complete study were prepared in one time and subsequently aliquoted and stored at -80 °C until further use. Prior to cell treatment, 10µM Aβ\textsubscript{oligo} or Aβ\textsubscript{fib} was incubated at RT for 1h alone or in combination with ACT (Calbiochem, Darmstadt, Germany), ApoE (rPeptide, Bogart, Georgia USA), ApoJ (isolated from human plasma by affinity chromatography as described \textsuperscript{44, 45}) (for all three peptides 1µM, 0.1µM and 0.01µM was used), 85 nM SAP (Calbiochem), 5nM C1q (purified in-house as described before \textsuperscript{17} or SAP and C1q together (SAP-C1q)).

**Astrocyte treatment**

Before treatment of the cells, astrocytes were plated in 24 well plates (NuncA/S, Roskilde, Denmark) at a density of 40,000 cells per well. Cells were allowed to adhere and reach 80% confluency, where after the various Aβ preparations (with or without AAPs) were applied for over-night incubation (18 hrs).

**RNA extraction and Reverse transcription**

Astrocytes were washed in PBS and homogenized using RNA-Bee solution (AMS Biotechnology, Abingdon, UK). The homogenates were collected and stored in sterile Eppendorf tubes at -20°C until total RNA extraction was performed. Total RNA was extracted using the RNeasy Mini kit (Qiagen GmbH, Hilden, Germany) in combination with DNase treatment, according to a protocol supplied by the manufacturer. Prior to storage at -80°C, RNA quantification was performed using the NanoDrop spectrophotometer (ThermoFisher, Wilmington, Delaware, USA). We normalized each treatment condition to total RNA input. Single stranded cDNA was synthesized using the High Capacity RNA-to-cDNA kit (Applied Biosystems; Foster City, California, USA) from 42 ng total RNA in a 20 µl reaction mixture according to protocol supplied by the manufacturer. All, cDNA samples were aliquoted and stored at -80°C until use.

**Quantitative real-time polymerase chain reaction (qRT-PCR)**

Real time-qPCR amplifications were performed using TaqMan\textsuperscript{*} Gene Expression Assays in optical 96-well reaction plates (Applied Biosystems) using an ABI 7500 Real Time PCR system with the SYBR Green\textsuperscript{*} Dye detection system. The following Taqman\textsuperscript{*} Gene Expression Assays with FAM labelled nucleotides were used (all Applied Biosystems): NEP (Assay ID: Hs00153510\textsubscript{m1}), IDE (Hs00610452\textsubscript{m1}), MMP-9 (Hs00189742\textsubscript{m1}), SCARB1 (Hs00194092\textsubscript{m1}), LRP2 (Hs00189742) and MARCO (Hs00198937\textsubscript{m1}). VIC-labelled GAPDH was used as an endogenous control (Applied Biosystems) and run simultaneously with the selected gene expression assays (multiplexed). A 20 µl reaction mixture contained 1µl GAPDH primers (20x), 1µl gene specific primers (20x), 10 µl of Taqman mastermix (Applied Biosystems) and 8µl cDNA (10 ng). The thermal protocol consisted of 2 minutes (min) at 50°C and 10 min of polymerase activation at
95°C, followed by 40 cycles of denaturation at 95°C for 15 seconds and primer annealing and extension at 60°C for 1 min. Each sample was amplified in duplicate. The cycle threshold (Ct) value is defined as the number of cycles required for the fluorescent signal to cross the threshold. The comparative Ct method $2^{-\Delta\Delta Ct}$ was used for performing relative quantification analysis. Some genes did not reach the cycle threshold after 40 cycles, therefore a pre-amplification step of 14 cycles was included. cDNA, prepared as mentioned above from two cell lines (human glioblastoma-astrocytoma, U373, and macrophage like human monocytic leukemia cell line, THP-1) was used as positive control for the studied genes. LRP2 is expressed in human brain tissue, therefore cDNA, prepared from brain tissue homogenates of one AD patient (Braak 6C) and one control patient (Braak 1-0) was used as a positive control for LRP2 expression. cDNA was omitted in the negative control samples and substituted by RNase free H$_2$O.

**Statistics**

Results per individual astrocyte culture were normalized against medium (Figure 1) or Aβ (Figure 2) treatment condition. Results are expressed as means ± SEM and comparisons between the various treatments were assessed with a student t-test on normalized data sets. A p-value of <0.05 was considered to reflect statistical significance.

**RESULTS**

Basal expression of target genes

Our first goal was to examine the basal gene expression of potential Aβ-receptors: MARCO, LRP2 and SCARB1 and Aβ-degrading enzymes: NEP, IDE and MMP-9 in our primary human astrocytes cultures. SCARB1, NEP and IDE were easily detectable in our primary human astrocytes, whereas the gene expression levels of MARCO, LRP2 and MMP-9 were not. Only after including a pre-amplification step these genes reached threshold, however the expression remained low (data not shown). Next, MARCO, LRP2 and MMP-9 expression was determined in the U373 astrocytome and THP-1 monocyte-macrophage cell line. In parallel to their very low expression in primary human astrocytes, MARCO expression was undetectable in U373 cells although it was found in THP-1 cells (data not shown). LRP2 expression was found in RNA isolated from post mortem brain specimens, but not in U373 and THP-1 cells (data not shown). MMP-9 was expressed in both U373 and THP-1 cells (data not shown). Due to their low expression in primary human astrocytes, we could not detect these three genes in our experimental set-up without a pre-amplification step, therefore only results on NEP, IDE and SCARB1 gene expression levels are reported.

Next, we compared the basal gene expression levels of NEP, IDE and SCARB1 in medium treated astrocytes derived of control- and AD brain (n=4 each). No differences
were found in basal expression levels of these genes between control and AD astrocytes (p>0.05; Table 1). Our analyses also show that the relative gene expression levels of NEP are much lower compared to the expression levels of IDE which is expressed at a 180-fold higher level than NEP (Table 2; p<0.001).

Influence of Aβ treatment on gene expression
Oligomeric and fibrillar Aβ were shown to influence different processes in the brain before 17, 43, 52 and we have previously demonstrated that human astrocytes preferably take up Aβ_{oligo} over Aβ_{fib} 8. We therefore examined whether the expression levels of NEP, IDE and SCARB1 were altered when astrocytes were treated with oligomeric or fibrillar Aβ peptide and whether there is a difference in treatment response between astrocytes derived from control and AD brains. Surprisingly, both Aβ_{fib} and Aβ_{oligo} alone did not

Table 1. Demographics of cases included in this study

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Braak score</th>
<th>ApoE genotype</th>
<th>PMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non demented control</td>
<td>Male</td>
<td>79</td>
<td>1A</td>
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<tr>
<td>Non demented control</td>
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</tr>
<tr>
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<td>4/3</td>
<td>N.A.</td>
</tr>
<tr>
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<td>N.A.</td>
<td>3/3</td>
<td>N.A.</td>
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<td>92</td>
<td>5C</td>
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<td>92</td>
<td>5C</td>
<td>4/3</td>
<td>04:55</td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td>Female</td>
<td>86</td>
<td>4B</td>
<td>3/3</td>
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</tr>
<tr>
<td>Alzheimer's disease</td>
<td>Female</td>
<td>76</td>
<td>6C</td>
<td>4/3</td>
<td>05:30</td>
</tr>
</tbody>
</table>

1 Clinical diagnosis with neuropathological confirmation (no neuropathological confirmation available for cases indicated with #. Tissue from these cases was obtained at surgery).
2 PMD, post mortem delay time (hours: minutes). N.A. not applicable

Table 2. Basal mRNA expression of NEP, IDE and SCARB1 in astrocytes derived from control and AD brain.

<table>
<thead>
<tr>
<th></th>
<th>Control astrocytes</th>
<th>AD astrocytes</th>
<th>Fold increase in gene expression^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal expression NEP</td>
<td>13.9 (±1.5)</td>
<td>13.1 (±0.2)</td>
<td>1</td>
</tr>
<tr>
<td>Basal expression IDE</td>
<td>6.0 (±0.3)</td>
<td>5.9 (±0.6)</td>
<td>180</td>
</tr>
<tr>
<td>Basal expression SCARB1</td>
<td>6.9 (±0.5)</td>
<td>7.0 (±0.6)</td>
<td>90</td>
</tr>
</tbody>
</table>

Basal gene expression of NEP, IDE and SCARB1 in medium treated astrocytes (n=4 brain derived astrocyte cases in each group). Data depicted as mean ΔCt values (Ct_{gene} – Ct_{GAPDH}) of duplicate measures (mean ±SEM). ^1 one missing value in AD brain derived astrocytes. ^2 Fold change calculated based on the mean values of control and AD brain derived astrocytes together. Fold change in basal expression as compared to NEP basal expression.
significantly influence the expression of any of the selected genes which remained near baseline expression levels (Figure 1).

**Influence of AAPs on gene expression**

In a recent study we showed that AAPs can influence the aggregation state of Aβ, when examined using electron microscopy, but AAPs were also shown to modulate Aβ uptake by primary human astrocytes. In continuation of our earlier studies we now investigated the effect of Aβ and several AAPs (SAP-C1q, ApoJ, ApoE and ACT) on gene expression in astrocytes derived from control and AD brain. As can be seen in Figure 2 and Table 3, AAPs modulate gene expression more profoundly in control astrocytes compared to AD astrocytes. Astrocytes derived from control brain up-regulate their expression of NEP when exposed to Aβ_{oligo} with ApoJ (4.5 times higher; p=0.04), Aβ_{oligo} with ApoE (4.4 times higher; p=0.03) and Aβ_{fib} with ApoE (2.8 times higher; p=0.03) compared to Aβ treatment alone. On the other hand, IDE expression in control brain derived astrocytes was down-regulated by Aβ_{fib} with SAP-C1q (1.4 times

**Figure 1.** Effect of Aβ_{oligo} and Aβ_{fib} on NEP, IDE and SCARBI gene expression in astrocytes derived from control and AD brain. Gene expression of medium treated cells were set to one, after which fold change was calculated for Aβ treatment. Horizontal lines represent means of expression levels. No effect of Aβ_{oligo} or Aβ_{fib} was found on gene expression of NEP, IDE and SCARBI.
Figure 2. Influence of Aβ-AAPs on NEP, IDE, and SCARB1 gene expression. Results from Aβ-AAP treated astrocytes were compared to results from Aβ-treated astrocytes alone (set at fold change 1). Horizontal lines represent mean fold change in gene expression and significant differences are indicated with * (p<0.05).
Table 3. Overview effect Aβ and AAPs on NEP, IDE and SCARB1 gene expression (based on Figure 1 and 2).

<table>
<thead>
<tr>
<th>Control brain derived astrocytes</th>
<th>Aβ Oligomers</th>
<th>Aβ Fibrils</th>
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<tbody>
<tr>
<td></td>
<td>NEP</td>
<td>IDE</td>
</tr>
<tr>
<td>Aβ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ + SAP-C1q</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Aβ + ApoJ</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Aβ + ApoE</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Aβ + ACT</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>AD brain derived astrocytes</th>
<th>Aβ Oligomers</th>
<th>Aβ Fibrils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NEP</td>
<td>IDE</td>
</tr>
<tr>
<td>Aβ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ + SAP-C1q</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ + ApoJ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ + ApoE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ + ACT</td>
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</tbody>
</table>

↑ = Upregulation of gene by specific Aβ ± AAP treatment  
↓ = Downregulation of gene by specific Aβ± AAP treatment

lower; p=0.03) and Aβ<sub>olf</sub> with ACT (2 times lower; p=0.03). SCARB1 gene expression was up-regulated in control astrocytes when treated with Aβ<sub>olf</sub> with SAP-C1q (1.8 times higher p=0.02) and Aβ<sub>tub</sub> with ApoE (1.7 times higher; p=0.01). No such effects were seen in AD derived astrocytes. The only effect in AD brain derived astrocytes was the down-regulation of IDE by Aβ<sub>tub</sub> with ApoE (1.3 times lower; p=0.02).

**DISCUSSION**

In this study we observed that primary human astrocytes *in vitro* express mRNA of three receptors proposed to be involved in Aβ clearance: SCARB1, MARCO and LRP2 and three Aβ degrading proteases: NEP, IDE and MMP-9, although for some genes pre-amplification was needed to reach threshold levels. Interestingly, Aβ<sub>olf</sub> and Aβ<sub>tub</sub> in combination with several AAPs, but not Aβ<sub>olf</sub> and Aβ<sub>tub</sub> alone, are capable of modulating astrocyte gene expression. This modulation is different in astrocytes derived from brain tissue of non-demented control subjects than in astrocytes derived from and AD patients.
The importance of astrocytes in Aβ clearance from the brain has become evident from several immunohistochemical studies describing the presence of intracellular Aβ in human astrocytes. Furthermore, *in vitro* studies show that primary human astrocytes are capable of binding and ingesting Aβ, however little is known about the Aβ uptake and degradation mechanisms in human astrocytes.

Scavenger receptors participate in the binding and uptake of many substances, including Aβ. In this study we show that human astrocytes express mRNA for SCARB1 and MARCO, suggesting that human astrocytes have the potential to bind and ingest Aβ. Indeed, the SCARB1 receptor was shown to play an important role in binding and endocytosis of Aβ by adult mouse astrocytes, mouse microglia and leptomeningal smooth muscle cells. To the best of our knowledge, we show for the first time MARCO mRNA to be present in human astrocytes, even though the expression appears to be remarkably low. These results are in line with the findings of other studies showing that MARCO mRNA was almost absent from human brain and very low on human macrophages. In contrast to the human situation, expression of MARCO in rodent astrocytes and macrophages is much higher, suggesting species dependent expression profiles. The LRP2 receptor expression was also investigated as a possible Aβ receptor, since complexes of Aβ with ApoJ were shown to be taken up via the LRP2 receptor which mediates cellular endocytosis and degradation. Although we found LRP2 mRNA in human brain tissue (data not shown), extremely low LRP2 mRNA expression in cultured human astrocytes was found, which suggests that other brain cells, such as brain endothelial- and choroid epithelial cells, possibly express LRP2 and mediate the uptake of Aβ complexed to ApoJ in the brain.

Basal expression of SCARB1 was not different between control and AD brain derived astrocytes, suggesting that the intrinsic expression of SCARB1 in human astrocytes is not dependent on the state of the disease. However, when astrocytes were exposed to Aβ-AAPs, control astrocytes increased mRNA levels under some conditions, whereas no change in mRNA expression in AD astrocytes was found. These data suggest a possible compensatory mechanism for SCARB1 expression in control astrocytes by increasing SCARB1 expression upon Aβ-AAP encounter, whereas this compensatory mechanism seems to be absent in AD derived astrocytes.

Binding and uptake of Aβ by astrocytes thus appears to be very important, however degradation of the peptide is another crucial step in Aβ clearance. Since NEP, IDE and MMP-9 are proposed to play an important role in Aβ degradation, we examined the expression of these genes in astrocytes. Basal expression levels of NEP and IDE did not differ between control and AD astrocytes, again suggesting there are no intrinsic differences between cultured astrocytes from non-demented subjects and AD patients. It is important to note that IDE expression was higher compared to NEP, suggesting a more prominent role of IDE in Aβ degradation in human astrocytes *in vitro*. When astrocytes were exposed to Aβ and AAPs, NEP expression was increased...
in control astrocytes under specific conditions, whereas no differences were found in AD astrocytes. In contrast to NEP, IDE expression was reduced upon combined Aβ and AAP treatment, suggesting a shift in the relative contribution of the astrocyte derived enzymes in the presence of Aβ and AAPs. NEP and IDE can both act as intracellular and extracellular proteases. It has, however been postulated that intracellular Aβ breakdown is mainly determined by NEP \(^{58}\) and that IDE is the principal extracellularly acting protease \(^{59}\). It is tempting to speculate that Aβ in the presence of AAPs could increase intracellular Aβ degradation, whereas the extracellular degradation is reduced in human astrocytes.

In contrast to the combined treatment with Aβ and AAPs, treatment of primary human astrocytes with Aβ oligo or Aβ fib alone, did not elicit any change in the genes examined. In a previous study we have shown that although ACT in combination with Aβ elicited a strong increase and modulation of several genes in human astrocytes, little effect of Aβ treatment by itself was found \(^{60}\). Together with our present data, this implicates the importance of a possible pathogenic role of AAPs when associated with Aβ. Both NEP and IDE protein levels are strongly influenced by ApoE genotype \(^{61,62}\). By selecting mainly patients with an ApoE ε4 allele (ApoE4+), we tried to avoid possible confounding effect of ApoE genotype on the gene expression levels. In this study ApoE modulated the expression of all three genes in combination with either Aβ oligo or Aβ fib, whereas the other AAPs did not, which confirms the role of ApoE in AD pathology. Recently we have shown that several AAPs, such as ApoJ and ApoE, strongly reduced the number of Aβ positive astrocytes when treated with Aβ oligo \(^8\). This suggests that apolipoproteins prevent Aβ oligo from binding and subsequent uptake by the astrocytes. In this study no strong modulation of SCARB1 was found upon treatment with Aβ oligo in combination ApoE or ApoJ in either control or AD brain derived astrocytes. However if both patients groups are combined, a trend towards increased SCARB1 expression upon exposure to Aβ oligo and apolipoproteins can be seen (Figure 2). Moreover, ApoJ and ApoE caused more than four times increase in NEP expression upon treatment with Aβ oligo. This suggests that astrocytes try to compensate for the decrease in Aβ uptake, as induced by ApoJ and ApoE, by increasing SCARB1 and NEP expression.

Several studies have reported results on astrocytic Aβ-uptake, however most studies were performed on rodent astrocytes \(^{12}\). It is known that regulation of genes can be different among species \(^{57}\). Therefore, increased knowledge about the astrocytic Aβ clearance mechanisms in AD can be obtained by studying cultured human astrocytes. In the current study we were able to examine four control brain derived astrocyte cultures and four AD brain derived astrocyte cultures. Ideally several more astrocyte cultures should be used, but nevertheless with significant differences obtained already at such low number of cultures we put forward that our results clearly indicate differences in expression of several important Aβ-clearance related genes between AD astrocytes and control astrocytes when exposed to Aβ and AAPs. AD astrocytes seem to have lost the
capacity to regulate gene expression upon Aβ and AAP encounter, whereas control astrocytes are far more flexible and react to stimuli more avidly. Most likely, AD should be targeted at a very early stage in disease development as astrocytic Aβ clearance might be hampered when Aβ turns fibrillar and AAPs become associated with Aβ. In view of the currently ongoing clinical vaccine trials, which aim to promote Aβ clearance, more experimental studies on mechanisms underlying human astrocytic Aβ clearance are strongly warranted. Future studies combining gene expression analyses, determination of translated gene products (protein levels) and enzyme activity of Aβ-degradation related molecules are crucial for success in unraveling the mechanisms underlying astrocytic Aβ clearance.

In summary, the current study shows that primary human astrocytes in culture express SCARB1, MARCO, LRP2, NEP, IDE and MMP-9, all of which are considered to be involved in Aβ clearance. We conclude that apolipoproteins, in combination with Aβ, can regulate the gene expression of SCARB1, NEP and IDE in astrocytes derived from non-demented brains, but that this regulatory effect is absent in astrocytes derived from AD brain.

ACKNOWLEDGEMENTS

This work was financially supported by Stichting Dioraphte (RV), The Swedish Tegger Foundation (HMN) and the Swedish Dementia Foundation (HMN). We would like to thank Marlies Jacobs for excellent technical assistance and Fedor Denkers, Serge Smeets and Cees Oudejans for fruitful discussions and suggestions on technical aspects of the study. We acknowledge Hans Niessen and Nynke Hahn of the cardiovascular research group at Pathology, VUmc for the kind gift of ApoJ. We also acknowledge the Netherlands Brain bank for providing post mortem brain specimens.
REFERENCES


