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Effects of Alzheimer's peptide and α 1-antichymotrypsin on astrocyte gene expression

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Abstract

We employed gene array technology to investigate the effects of α 1-antichymotrypsin (ACT), soluble or fibrillar Alzheimer's peptide ($A\beta_{1-42}$) alone and the combination of ACT/ $A\beta_{1-42}$ on human astrocytes. Using a 1.2-fold change as significance threshold, 398 astrocyte genes showed altered expression in response to these treatments compared to controls. Of the 276 genes affected by the ACT/soluble $A\beta_{1-42}$ combination, 195 (70.6%) were suppressed. The ACT/fibrillar $A\beta_{1-42}$ combination affected expression of 64 genes of which 58 (90.5%) were up-regulated. The most prominent gene expression changes in response to the ACT/soluble $A\beta_{1-42}$, were the down-regulation of at least 60 genes involved in transcription, signal transduction, apoptosis and neurogenesis. The ACT/fibrillar $A\beta_{1-42}$ increased the expression of genes involved in transcription regulation and signal transduction. Surprisingly, gene expression of astrocytes exposed to soluble or fibrillar $A\beta_{1-42}$ alone was largely unaffected. Thus, the molecular forms generated by the combination of ACT/ $A\beta_{1-42}$ alter expression of astrocyte genes more profoundly in breadth and magnitude than soluble or fibrillar $A\beta_{1-42}$ alone, suggesting that pathogenic effects of $A\beta_{1-42}$ may occur as a consequence of its association with other proteins.

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Keywords: Alzheimer's peptide ($A\beta_{42}$); Astrocytes; α 1-Antichymotrypsin; Alzheimer's disease; Gene expression; Inflammation

1. Introduction

Both sporadic and familial Alzheimer's disease (AD) are characterized by prominent neuroinflammation and

senile/neuritic plaques, mainly composed of aggregated amyloid-beta ($A\beta$) peptides, in proximity to activated microglia, astrocytes and neurons [25,26,43,48]. The various length amyloid peptides ($A\beta_{1-40}$, $A\beta_{1-42}$) are formed by β - and γ -secretase degradation of amyloid precursor protein (APP) [7], are secreted by different types of cells during normal metabolism [8] and can be detected in plasma, cerebrospinal fluid and brain from normal and AD patients [35,41].

$A\beta$ has been suggested to play a role in AD through its interaction with other molecules by creating complexes or new molecular forms that gain biological activities or lose essential regulatory activities, thereby fostering chronic inflammation. In vivo, $A\beta$ exists in complex with other molecules like apolipoproteins E and J, α 1-antichymotrypsin,

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amyloid P component, cytokines, complement and extracellular matrix proteins, most of which also occur in amyloid deposits [9,44,47]. These other molecules may chaperone A β self-association and lead to formation of toxic A β oligomers that are diffusible and potentially more pernicious than fibrillar A β .

A link between AD and α 1-antichymotrypsin (ACT), a serine proteinase inhibitor, was established through observations that ACT is found in both amorphous and classical plaques of AD [2], and in the amyloid deposits of Down Syndrome and normally aged brains [29]. ACT is found to co-localize specifically with A β _{1–42} deposits, and also to be highly expressed in areas of brain that are prone to plaque development [21]. Through use of monoclonal antibodies to the different forms of ACT, it has been shown that ACT in both amorphous and classic plaques is either complexed or proteolytically cleaved [38]. In vivo overproduction of ACT by astroglial cells has been described, and ACT levels in the brain were found to correlate with the number of activated astrocytes [1]. Recent in vivo studies using transgenic mice models expressing astrocytic ACT, have shown that ACT promotes plaque deposition in brain tissue [28,32] and it has also been demonstrated that cognitive impairment in an APP mouse model is dependent on ACT-catalysed amyloid formation [31]. We have proposed that the interaction between A β and ACT results in loss of inhibitor activity of ACT [14] and creates molecular forms with new physiological activities that could promote chronic inflammation and amyloid formation in AD [16].

The present study was conducted to test the hypothesis that different cellular responses at the level of gene expression are elicited by various forms of A β . Our aim was to obtain and compare gene expression profiles in primary human astrocytes exposed to either ACT or soluble and fibrillar A β alone, and to ACT/A β combinations.

2. Materials and methods

2.1. α 1-Antichymotrypsin

Human plasma α 1-antichymotrypsin (Calbiochem, La Jolla, USA) was reconstituted in sterile 0.9% NaCl (Fresenius Kabi, Uppsala, Sweden) and decontaminated of endotoxin with Detoxi-Gel AffinityPak columns, according to manufacturer's instructions (Pierce, IL, USA). Purified batches of ACT were checked for endotoxin with the Limulus amoebocyte lysate endochrome kit (Charles River Endosafe, SC, USA) and endotoxin levels were less than 0.14 enzyme units/mg protein in all ACT preparations used. The ACT protein concentration was determined using the Lowry method [22] and protein quality was analysed by 7.5% SDS-PAGE electrophoresis.

2.2. Lipopolysaccharide

Lipopolysaccharide (LPS) from *E. coli* serotype 026:B6, Lot 17H4042 (Sigma, Steinheim, Germany) was reconstituted in sterile 0.9% NaCl.

2.3. A β _{1–42} peptide

Synthetic A β _{1–42} peptide was obtained from Saveen (Denmark), >95% pure, M_w = 4512.9, and from Oncogene (San Diego, USA) and Calbiochem >95% purity, M_w = 4515. The peptide was reconstituted in sterile 0.9% NaCl and aliquots to be used as a soluble A β _{1–42} peptide were either used immediately or immediately frozen at -156°C until used. For fibril formation, the A β _{1–42} solution was kept for 3 days under sterile conditions at room temperature [15].

2.4. Electron microscopy

Aliquots of soluble and fibrillar A β _{1–42} alone and in combination with ACT (at a molar ratio of 10:1 A β _{1–42}:ACT) were applied to carbon-coated copper grids, negatively stained with 2% (w/v) uranyl acetate or phosphotungstic acid and visualized in a JEOL 200 cx operating at 80 kV.

2.5. Astrocyte culture

Primary human astrocytes (BioWhittaker, Walkersville, USA) were seeded in 25 cm² TC Nunclone™ Delta Surface flasks (Nunc A/S, Roskilde, Denmark) and grown in astrocyte basal medium (ABM™) supplemented with AGM™ SingleQuots® (Cambrex, Walkersville, USA) containing fetal bovine serum, ascorbic acid, rhEGF, GA-1000, insulin and L-glutamine. Cells were maintained at 37 °C in an atmosphere of humidified air with 5% CO₂. Confluent cultures of astrocytes were harvested using ReagentPack™ (Cambrex) including trypsin neutralising solution, trypsin/EDTA and HEPES buffered saline solution.

2.6. Astrocyte treatment

Astrocytes were used after reaching confluency, 3–4 days after culture in Nunclone™ Delta Surface 80 cm² flasks (Nunc A/S). Prior to experiments, cell-culture medium was changed to serum-free medium and cells were stimulated with LPS (10 $\mu\text{g}/\text{mL}$), ACT (0.6 μM), soluble or fibrillar A β _{1–42} (2.6 μM), ACT/A β _{1–42} combinations or to an equivalent volume of phosphate buffered saline (PBS) for 18 h at 37 °C. Experiments were performed in triplicate.

2.7. RNA preparation and gene expression profiling by Affymetrix GeneChip microarrays

Total RNA from three cell culture experiments was prepared using TRIZOL® (GibcoBRL Invitrogen, Stockholm, Sweden). RNeasy Kit (VWR International, Stockholm, Swe-

Table 1
Primers used in SYBR green quantitative PCR

Gene	Primers	
	Sense	Antisense
MCP-1	ATAGCAGCCACCTTCATTCC	ATAACAGCAGGTGACTGGGG
SOD2	AATCAGGATCCACTGCAAGG	GGTAGTAAGCGTGCTCCAC
CRMP2	AAGCGTATCAAGGCAAGGAG	TTCACACACAGGTCCGTCAT
Thioredoxin reductase 1	AGCAGCTGGACAGCACAA	TAACCTCAGCAGCCAGCC
Proteasome component	GCAGCCTCCATTTTACAGGA	TAGGCAGAGCCTCATTTGCT
HIP2	CGTCACAGGGGCTATTTGTT	TGACAATAATACCGTGCGGA
Numb	ATCTGTCACTGCTTCATGGCT	CTAAACAGGCTGCAAAAGCAC

MCP-1, monocyte chemoattractant protein-1; SOD2, superoxide dismutase 2; CRMP2, Collapsin response mediator protein 2; HIP2, huntington interacting protein 2.

den) was used to purify the isolated RNA. Prior to cDNA synthesis the quality and concentration of RNA was determined using the Agilent Bioanalyser 2100 and Nanodrop. Five micrograms of total RNA from each sample was used to generate a high-fidelity cDNA, according to routine protocols at Swegene, Microarray Resource Centre. Briefly, double-stranded cDNA was synthesized according to the Affymetrix GeneChip Expression Analysis Technical Manual. This cDNA was then used in an in vitro transcription reaction in the presence of biotinylated nucleotides using Enzo BioArray™ HighYield™ RNA Transcript Labelling Kit (Affymetrix). Twenty micrograms of full-length cRNA was fragmented and subjected to gene expression analysis on Affymetrix U133A, high-density oligonucleotide array representing 18,400 transcripts. Hybridization, staining and washing of all arrays were performed according to the protocol provided by Affymetrix (www.affymetrix.com).

2.8. Microarray data analysis

Probe level expression values from each test RNA pool were analysed using dCHIP (www.dchip.org) and modelled using the perfect match mismatch algorithm [20]. Each sample in the dataset was normalized to a median baseline array with a mean intensity determined by the dChip software (actual value 221). The resulting expression signals were used to calculate fold changes between basal and experimental conditions. Only genes showing a minimum of a 1.2-fold change (lower bound of the confidence interval for the fold change) and a minimum intensity difference of 25 in all three experiments were selected. An unpaired *t*-test ($p=0.05$) was performed alongside to rank these genes rather than using it as an actual significance value. Functional categorization of genes over-represented or suppressed was performed using the Expression Analysis Significance Explorer (EASE) program downloaded from the NIAID web site (<http://apps1.niaid.nih.gov/David/upload.asp>).

2.9. Quantitative real time PCR

Primers for selected genes were designed using the Stratagene labtools software (www.labtools.stratagene.com) and

are presented in Table 1. RNA was converted into cDNA using Stratascript reverse transcriptase (Stratagene). Approximately 50 ng of the resulting cDNA was subjected to real time PCR using 2X Brilliant SYBR green mastermix (Stratagene) and 150 nM of each primer. These reactions were performed in duplicate on a MX3000 thermocycler (Stratagene) with an annealing temperature of 60 °C. The resulting product sizes were confirmed on a 2% agarose gel and a dissociation curve analysed to ensure specific product amplification and to eliminate contribution from primer dimer. All assay efficiencies were monitored using a standard curve. The resulting cTs were normalized to the thioredoxin reductase 1 house-keeper gene, which showed no expression changes on the microarray following any of the treatments. The relative fold change compared to the basal sample was calculated using the $\Delta\Delta C_T$ method ($2^{-\Delta\Delta C_T}$) [23].

2.10. MCP-1 analysis

Cell culture supernatants from astrocytes treated with ACT, LPS, soluble or fibrillar A β_{1-42} or ACT/A β_{1-42} combinations were analysed to determine MCP-1 levels. A quantitative enzyme linked immuno-sorbent assay (ELISA) (R&D Systems, Minneapolis, USA) was used according to manufacturer's instructions. The optical density was determined using a microplate reader at 450 nm and readings at 570 nm were subtracted from the readings at 450 nm for wavelength correction. The duplicate readings for each standard, control and sample were averaged and the optical density of the average zero standard was subtracted.

3. Results

3.1. Characterization of A β_{1-42} alone and in mixture with ACT by electron microscopy

Preparations of soluble (freshly prepared) and fibril (pre-incubated for 3 days at room temperature) A β_{1-42} , alone and in mixture with ACT at a molar ratio of 10:1 (A β_{1-42} :ACT), were examined by electron microscopy. ACT/A β_{1-42} mixtures were examined after 18 h incubation

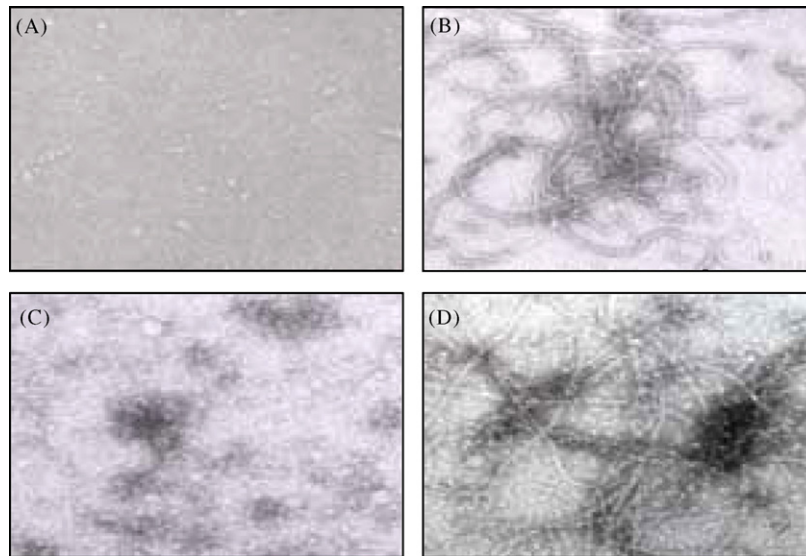


Fig. 1. Electron micrographs of the structures formed by synthetic $A\beta_{1-42}$ peptide in the absence and presence of ACT in a molar ratio of 1:10 ACT: $A\beta_{1-42}$. (A and B) $A\beta_{1-42}$ peptide freshly dissolved and $A\beta_{1-42}$ peptide incubated for 3 days at room temperature, respectively. (C and D) Soluble and fibrillar $A\beta_{1-42}$ in the presence of ACT after 24 h at 37 °C, respectively.

at 37 °C. Fig. 1A–D represents typical profiles of samples taken from $A\beta_{1-42}$ preparations alone or mixed with ACT. As illustrated (Fig. 1A), soluble $A\beta_{1-42}$ alone manifests no fibril or aggregate formation. However, when both soluble $A\beta_{1-42}$ and ACT are combined, a large number of amorphous aggregates varying in size occur in the sample (Fig. 1C). Pre-incubation of the $A\beta_{1-42}$ peptide for 3 days results in the formation of variable length fibrils (Fig. 1B). Mixtures of ACT/fibrillar $A\beta_{1-42}$ contain a large number of protein aggregates and fibrils (Fig. 1D).

3.2. Gene expression profiles of human astrocytes that are exposed to ACT and soluble or fibrillar $A\beta_{1-42}$ separately and in combination

Primary human astrocytes were treated with ACT, soluble or fibrillar $A\beta_{1-42}$ alone, or their combinations (ACT/ $A\beta$) for 18 h. Non-stimulated cells were used as negative controls. In addition, cells were treated with LPS, which induces pro-inflammatory activation of various cell types. All treatments ($n=7$) were performed in triplicate resulting in 21 chips of data. The data were analysed using dChip, software designed for oligonucleotide array analysis [20]. The arrays were of good quality with a low percentage of outliers and a high percentage of present calls, the only exception was the ACT/fibrillar $A\beta$ array from the first experiment (16% outliers), which was excluded from further analysis. Differential gene expression in treated cells was determined using a cut-off of 1.2 in fold change. This is not an arbitrary choice and does indeed reflect a 20% change and it is stringent. This is a conservative estimate as it represents the lower boundary of the confidence interval of the fold change for three separate arrays [19]. With these criteria, a total of

398 genes showed a difference in expression in treated conditions compared to basal levels. Among these 398 genes 172 were up-regulated and 226 were down-regulated. Treatment of cells with ACT/soluble $A\beta$ significantly affected a total of 276 genes, of which 70.5% were down-regulated, while ACT/fibrillar $A\beta$ treatment affected 64 genes, 90.5% of which were up-regulated (Fig. 2). Nearly all of these gene

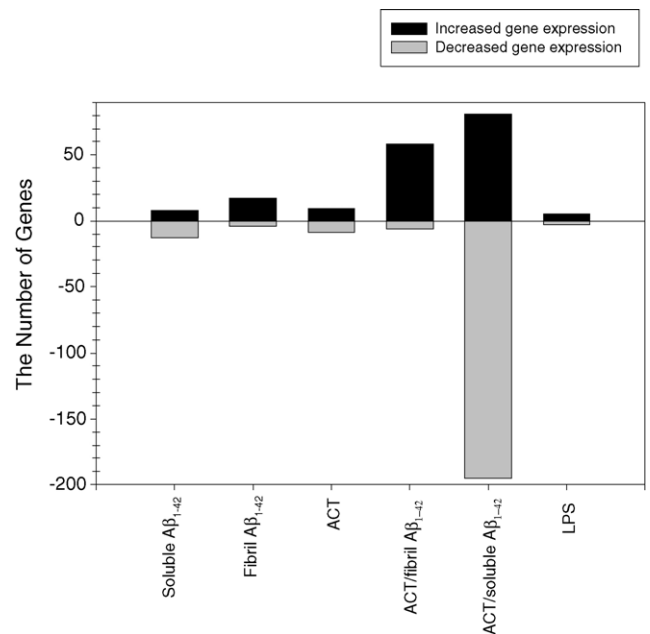


Fig. 2. Summary of the total number of up-regulated and down-regulated genes in astrocytes. Primary human astrocytes were stimulated with soluble or fibrillar $A\beta_{1-42}$ alone or in the presence of ACT and with ACT and LPS alone for 18 h vs. unchallenged cells. A gene expression change of 1.2-fold or greater over the unchallenged astrocytes was considered to be significant.

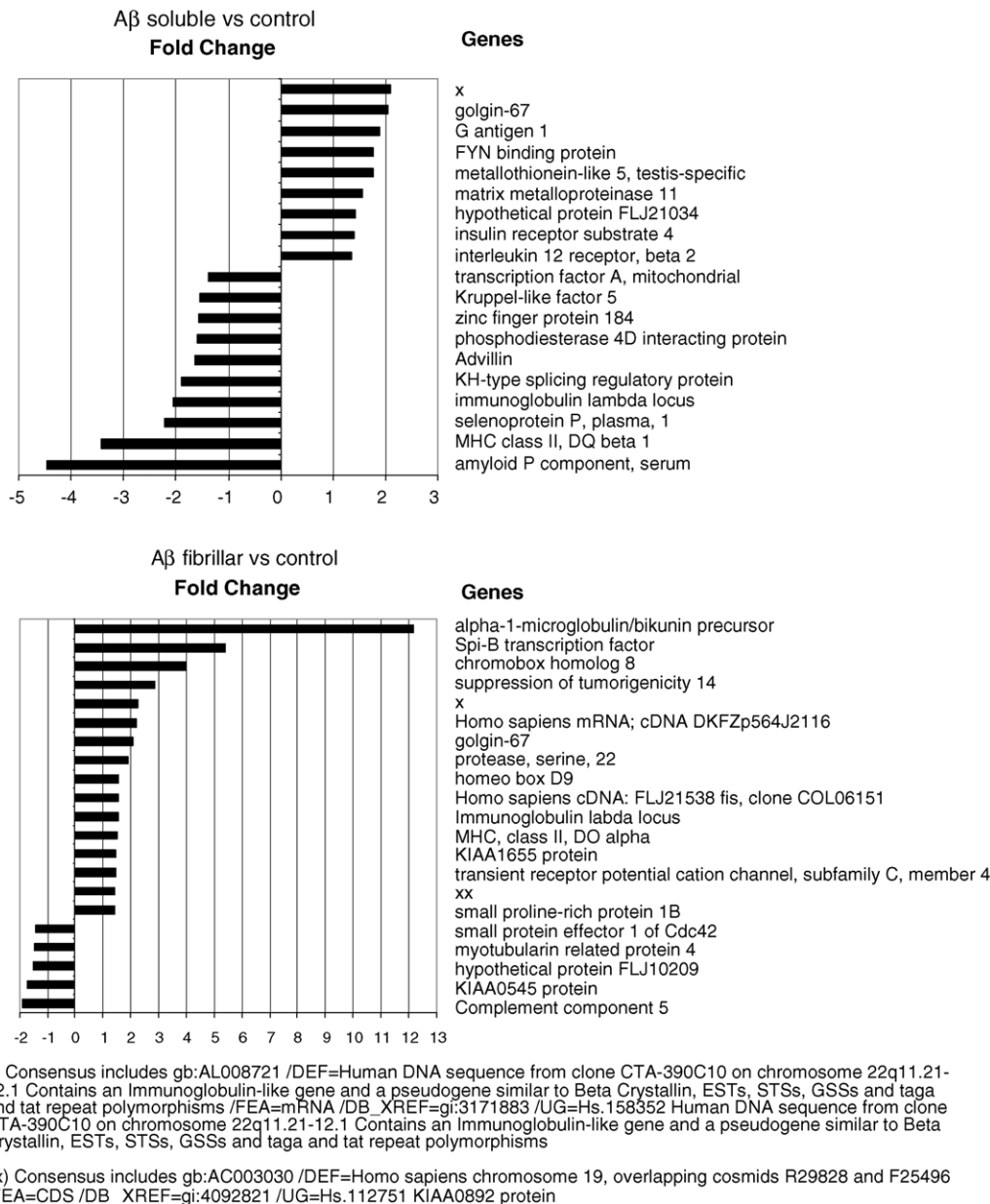


Fig. 3. A bar graph showing the genes affected by the Aβ soluble or fibrillar single treatments. All genes are showing a 1.2-fold change compared with basal.

expression changes were unique to the combined ACT/Aβ treatments, with only 4% of the ACT/soluble Aβ genes and 3% of the ACT/fibril Aβ genes overlapping with the Aβ or ACT treatments alone. As shown in Fig. 3, soluble and fibrillar Aβ peptide alone affected expression of a total of 19 and 21 genes, respectively. Of these affected genes 62% were down-regulated by soluble Aβ, whereas 81% were up-regulated by fibrillar Aβ. It is noteworthy that treatment of cells with ACT alone affected only 18 genes compared to non-treated controls (Fig. 4). Unexpectedly, expression of only eight genes was affected by LPS treatment of astrocytes, of which five were up-regulated and three down-regulated (Fig. 5).

3.3. Differences in gene expression profiles of human astrocytes treated with the ACT/soluble Aβ and ACT/fibril Aβ combinations

A broad functional classification by biological process of the genes whose expression was altered in treated astrocytes compared to controls is given in Fig. 6. Specific terms for these processes were extracted from multiple levels within the gene ontology hierarchy, so that only major gene categories are listed. This broad classification includes genes encoding molecules for signal transduction, transcription, response to stress, neurogenesis and others. Approximately 30% of the analysed genes have unknown function and could not be

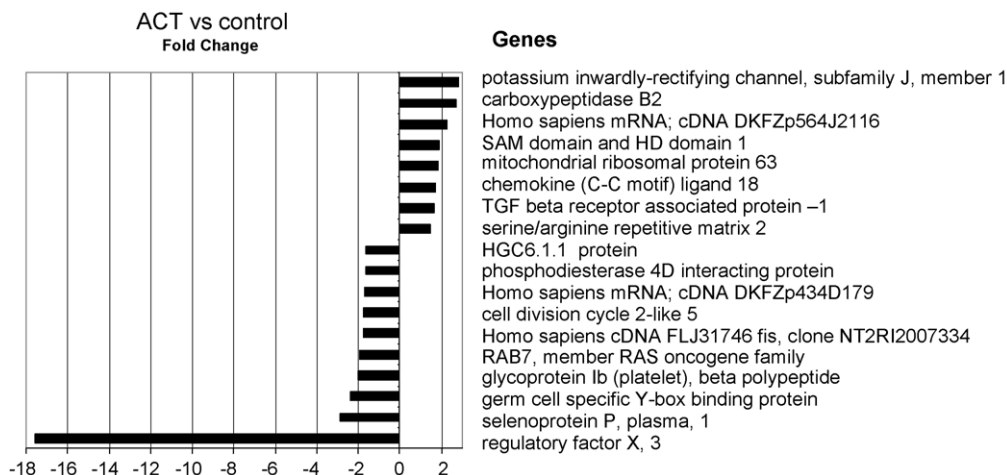


Fig. 4. A bar graph showing the genes affected by the ACT treatment. All genes are showing a 1.2-fold change compared with basal.

classified. As shown in Fig. 6 the treatment of astrocytes with ACT/soluble A β combination altered the expression of 40 genes involved in transcriptional regulation, 18 genes involved in signal transduction, 12 in apoptosis and 9 in neurogenesis. Interestingly, the ACT/fibrillar A β combination showed much less pronounced effect on the expression of genes involved in transcription and signal transduction and had no effect on genes involved in apoptosis or stress response.

Table 2 lists detailed expression profiles for these major gene categories and shows a striking preponderance of decreased transcript expression in a number of classes of the genes in response to ACT/soluble A β in contrast to the response to ACT/fibrillar A β combination, in which increased transcript expression was observed for a large majority of the affected genes. Only two common genes were affected by the ACT/soluble A β and ACT/fibrillar

A β treatments using our selection criteria: ubiquitin specific protease 12 decreased -1.79 - and -1.93 -fold and suppression of tumorigenicity 14 increased 2.49 - and 2.4 -fold, respectively.

3.4. Verification of the specific astrocyte genes affected by the LPS and ACT/A β treatments by quantitative real time PCR

Treatment of astrocytes with ACT/soluble A β combination decreased the expression of two genes involved in neurogenesis: Numb and Collapsin response mediator protein 2 (CRMP2) also known as dihydropyrimidinase-like 2 [33]. This treatment also decreased the expression of a number of genes involved in the ubiquitin and protein degradation system. These included the proteasome (prosome, macropain) 26S subunit, non-ATPase, 12, a ubiquitin ligase, cullin 4A

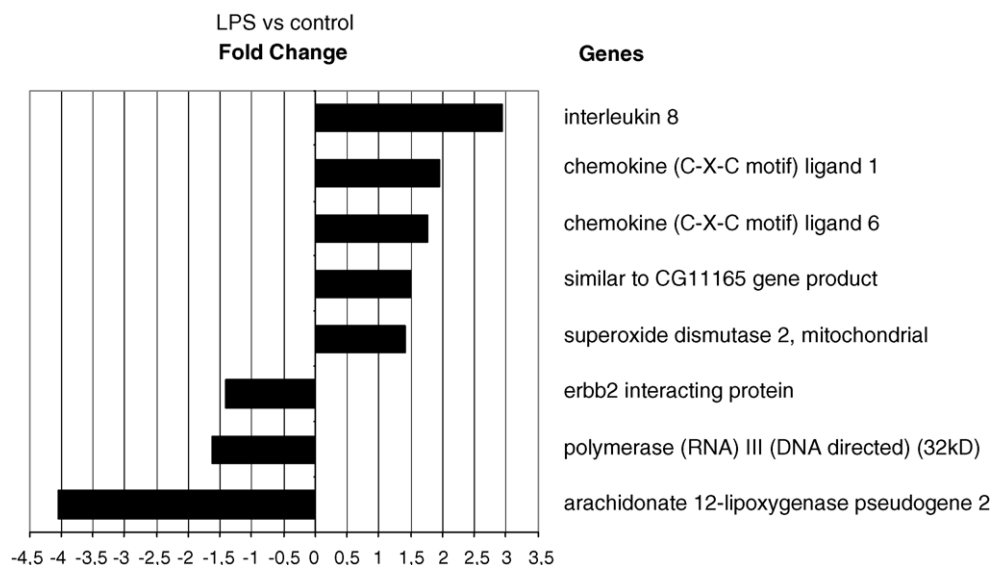


Fig. 5. A bar graph showing the genes affected by the LPS treatment. All genes are showing a 1.2-fold change compared with basal.

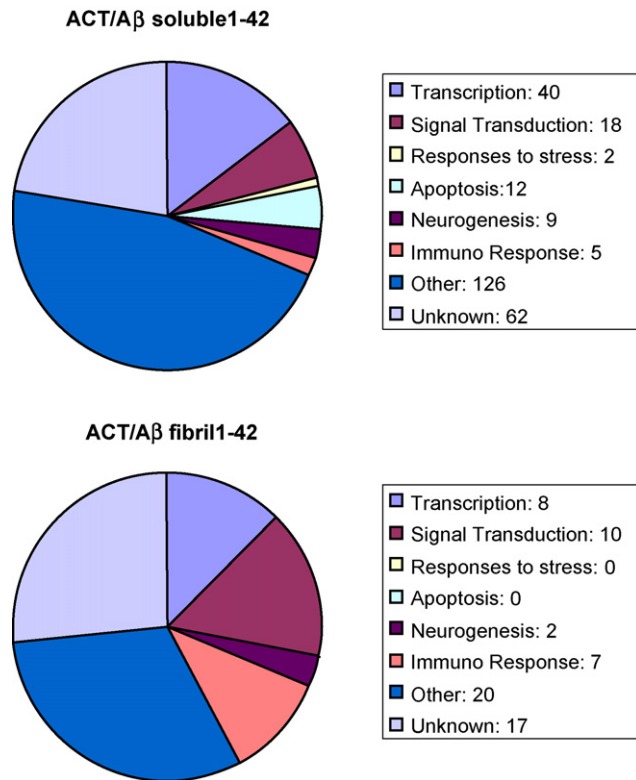


Fig. 6. A broad functional classification of the genes whose expression was altered in astrocytes exposed to ACT/soluble Aβ₁₋₄₂ and ACT/fibril Aβ₁₋₄₂ compared to un-treated cells.

and the ubiquitin conjugating enzymes E2D 1 and huntingtin interacting protein 2.

Exposure of cells to combined ACT/fibrillar Aβ resulted in increased expression of inflammatory response genes, such as chemokine ligands 1, 6 and 24, chemokine receptor 1, monocyte chemotactic protein 1 (MCP-1) and interleukin 1 receptor antagonist. Moreover, transcript expression of the superoxide dismutase 2 (SOD2) gene is increased by endotoxin, suggesting that the oxidative stress pathway is involved in endotoxin-mediated astrocyte activation. In order to verify the data obtained from the microarray analysis, SYBR green

assays were designed for MCP-1, SOD2, CRMP2, Numb, HIP2 and a proteasome component. The expression profiles of these selected genes from the microarray were successfully replicated using real time PCR (Fig. 7). The fold changes observed with real time PCR were approximately double the array fold changes, but in many of instances the direction of the change matched. As illustrated in Fig. 8, exposure of astrocytes to ACT, Aβ₁₋₄₂ and ACT/Aβ₁₋₄₂ combinations induced changes in MCP-1 release in a similar manner as observed by microarray analysis and real time PCR.

4. Discussion

In this study microarrays were used to evaluate the gene expression profiles in cultured primary human astrocytes exposed to ACT and to various forms of Aβ₁₋₄₂ alone and in combination with ACT. LPS was used to mimic the pro-inflammatory conditions seen in AD brain. To ensure that the microarray generated reliable data, expression changes of a selected number of genes were successfully verified using real time PCR and protein release of MCP-1. Our results reveal that exposure of astrocytes to either soluble or fibrillar Aβ₁₋₄₂ peptide alone lead to changes in expression of a remarkably small set of genes. Interestingly, these two forms of Aβ₁₋₄₂ have generally contrasting effects on the direction of the gene expression changes. The soluble Aβ₁₋₄₂ form leads to a larger proportion of genes with negative expression changes (e.g. amyloid P component, MHC, class II and selenoprotein P), whereas fibrillar Aβ₁₋₄₂ increases the expression of more genes than it decreases (e.g. alpha1-microglobulin, MHC class II and genes involved transcription regulation). The only gene affected by both soluble and fibrillar Aβ₁₋₄₂ is Golgin 67, whose expression is increased by exposure to both forms of Aβ₁₋₄₂. Golgin 67 is a novel Golgi protein with a C-terminal transmembrane domain. The structural and sequence similarities of Golgin 67 to other Golgi proteins suggest that, in addition to a possible involvement in the Golgi apparatus organization, this protein may also play a role in docking/tethering of vesicles [13].

Table 2

Distribution of differentially expressed genes based on the major biological processes as defined by the GO consortium (<http://www.geneontology.org/>)

Gene classification	Aβ ₁₋₄₂ soluble		Aβ ₁₋₄₂ fibril		ACT		ACT/Aβ ₁₋₄₂ soluble		ACT/Aβ ₁₋₄₂ fibril	
	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
Transcription		3	3			1	4	36	6	2
Signal transduction	2			1		1	4	14	10	
Response to stress		1				1		2		
Apoptosis	1						3	9		
Neurogenesis		1					4	5		2
Immune response	1	1	2	1	2		4	1		7
Other ^a	3	3	5	2	5	4	34	92	19	1
Unknown ^b	2	1	6	1	1	3	28	34	14	3
Total number	9	10	16	5	8	10	81	195	58	6

^a Do not fit into any of the above classification.

^b No function yet assigned.

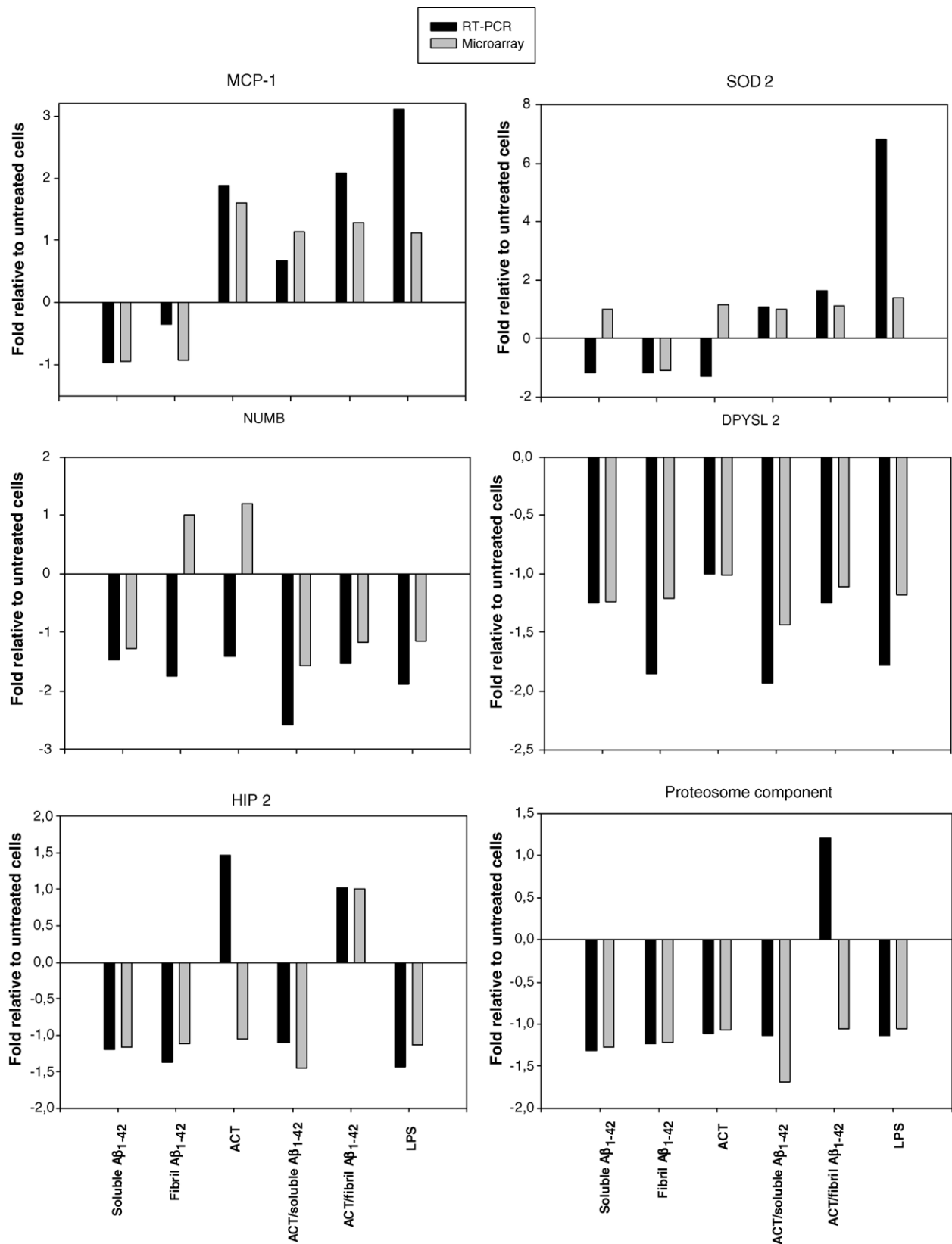


Fig. 7. The evaluation of mRNA levels for a subset of genes using semi-quantitative RT-PCR and comparison to microarray data. Data are presented as fold relative to untreated cells.

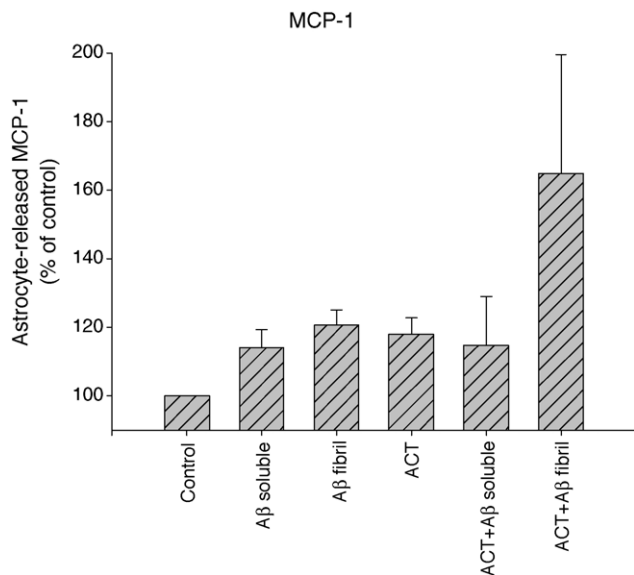


Fig. 8. MCP-1 release from astrocytes stimulated with soluble or fibril A β _{1–42} alone or in combination with ACT and ACT alone. Each bar represents the mean \pm S.D. of three separate experiments.

Lipopolysaccharide is known to activate the innate immune system, triggering gliosis and inflammation when injected into the central nervous system. Recent studies by Herber et al. with APP transgenic mice have shown that injected intra-hippocampally, LPS activates both microglia and astrocytes and significantly reduces diffuse A β deposits, but has no effect on compacted fibrillar amyloid [10]. It is interesting to note that astrocyte treatment with LPS affected expression of only eight genes relative to basal levels. The most significantly up-regulated genes were found to be IL-8, monocyte chemoattractant protein-1 and granulocyte chemotactic protein 2. Future experiments are needed to ascertain the link between LPS-induced chemokine activation in astrocytes and A β .

Our experiments were designed to model the influence of fibrillar or soluble A β _{1–42} in the presence of ACT on astrocyte gene expression at the transcriptional level. Our data for the first time shows that the combined treatment of both A β and ACT induces expression of approximately six times more genes than treatments with each of these reactants alone. This indicates the existence of new molecular species deriving from the ACT/A β _{1–42} combinations which have distinct effects on transcriptional gene expression. Furthermore, the effects of the combinations of ACT with soluble or fibrillar A β _{1–42} on gene expression numbers were markedly different. For example, ACT/soluble A β _{1–42} affected about four times more genes than the ACT/fibrillar A β _{1–42} combination and 70.6% of affected genes in the former were down-regulated. In contrast, the ACT/fibrillar A β _{1–42} combination up-regulated 90% of the 64 genes significantly affected by it. These contrasting effects of ACT/soluble and ACT/fibrillar A β _{1–42} were particularly pronounced in gene groups involved in transcription and sig-

nal transduction, and could thus have profound biological effects.

The A β peptide has been inferred to exert its neurotoxic effects by activating inflammatory pathways [3] and by enhancing oxidative mechanisms that lead to oxidative stress [49] and induced apoptosis [36]. To follow up the array results, the expression of certain genes suggested to be involved in A β neurotoxic effects was examined by RT-PCR methods. Among the genes similarly affected in astrocytes stimulated with ACT/soluble or ACT/fibrillar A β _{1–42}, the Notch antagonist Numb, which has a role in cell fate determination, and is vital for neurogenesis [6], was an obvious candidate of significance. Numb interacts with proteins involved in ubiquitination [17] [27,30,45] and endocytosis [4,39,42] and is thought to act as an adaptor protein for protein degradation [12]. Interestingly, Numb interacts with CRMP2 during endocytosis [33] and both genes show a decrease in expression with the ACT/A β treatments. CRMP-2 has been found to be highly oxidised in AD brains compared to controls [5] and down-regulated in AD and Down syndrome's brains [24]. We hypothesize that the decreased expression of Numb and CRMP2 caused by ACT/A β complexes may be a consequence of importance for the etiology of AD. Of particular relevance, the phosphotyrosine domain of Numb has been shown to bind to the YENPTY motif within the intracellular domain of APP [37]. This intracellular domain of APP is also capable of inhibiting Notch through Numb, providing a direct link between development and neurodegeneration [40]. There is further evidence from our data that the ACT/A β treatment is up regulating the Notch pathway. Hairy, a transcription factor targeted by the Notch pathway, shows increased transcriptional expression with ACT/fibrillar A β treatment. This is the first evidence that A β -ACT treatment of astrocytes can affect genes involved in the Notch pathway.

There is much evidence that inflammation is involved in the pathology of Alzheimer's disease [3]. The presence of an amyloid plaque triggers an inflammatory response in the brain, similar to what one would expect from the presence of abnormal proteins within the periphery, but the timing and role of inflammation and its contribution to the onset of Alzheimer's disease is still being debated. ACT/fibrillar A β caused an increase in the expression of six chemokine genes that may be responsible for the movement of astrocytes and glial cells toward the senile plaque. As noted above, MCP-1 is of particular interest, as it has previously been reported to show an increase with A β stimulation in primary human astrocytes [43] and post mortem microglia [46] and is also over-expressed on exposure of astrocytes to the unrelated pro-inflammatory agent, LPS.

Oxidative stress is also widely implicated in the development of neurodegenerative diseases including Alzheimer's [18]. We have shown that ACT/fibrillar A β treatment causes an increase in the expression of SOD2, a gene implicated in suppression of oxidative stress. It has previously been reported that SOD2 expression is increased in the senile plaques of Alzheimer's diseased brains [34]. SOD2 null mice are a

model for exogenous oxidative stress and have a severe neurological phenotype [11].

Our results demonstrate a complex alteration of gene expression following astrocyte stimulation with either A β alone or in combination with ACT and strongly suggest that ACT plays an important role in modulating A β effects on human astrocyte activation. The central role of ACT in the inflammatory responses provides a link between these pathways and AD, suggesting that ACT may be as important as A β in the etiology of the disease.

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