Amyloid-β Oligomers Induce Differential Gene Expression in Adult Human Brain Slices

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Cognitive decline in Alzheimer disease (AD) is increasingly attributed to the neuronal impact of soluble oligomers of the amyloid-β peptide (AβOs). Current knowledge on the molecular and cellular mechanisms underlying the toxicity of AβOs stems largely from rodent-derived cell/tissue culture experiments or from transgenic models of AD, which do not necessarily recapitulate the complexity of the human disease. Here, we used DNA microarray and RT-PCR to investigate changes in transcription in adult human cortical slices exposed to sublethal doses of AβOs. The results revealed a set of 27 genes that showed consistent differential expression upon exposure of slices from three different donors to AβOs. Functional classification of differentially expressed genes revealed that AβOs impact pathways important for neuronal physiology and known to be dysregulated in AD, including vesicle trafficking, cell adhesion, actin cytoskeleton dynamics, and insulin signaling. Most genes (70%) were down-regulated by AβO treatment, suggesting a predominantly inhibitory effect on the corresponding pathways. Significantly, AβOs induced down-regulation of synaptophysin, a presynaptic vesicle membrane protein, suggesting a mechanism by which oligomers cause synapse failure. The results provide insight into early mechanisms of pathogenesis of AD and suggest that the neuronal pathways affected by AβOs may be targets for the development of novel diagnostic or therapeutic approaches.

Alzheimer disease (AD),5 the most common form of dementia in the elderly, currently affects more than 35 million people worldwide (1). AD is characterized clinically by early memory dysfunction followed by progressive cognitive impairment (1, 2). Soluble oligomers of the amyloid-β peptide (AβOs) are currently considered central players in the pathogenesis of AD (3–6). AβOs initially were found to act as potent cell surface ligands, instigating rapid inhibition of long-term potentiation and alterations in neuronal cell signaling (7). Those findings have been followed by reports on a variety of neurotoxic effects of AβOs, including Tau hyperphosphorylation (8), calcium dysregulation and oxidative stress (9–13), blockade of fast axonal transport (14, 15), altered turnover of neuronal receptors involved in synaptic plasticity (e.g. 16–20), and synapse loss (17, 19).

Although it is now generally accepted that AβOs cause synapse dysfunction, the molecular and cellular mechanisms underlying their toxicity are still poorly understood. In part, this is because most studies of AβO toxicity have utilized rodent-derived cell/tissue cultures or transgenic models of AD, which do not necessarily recapitulate the complexity of the human disease. Previous studies have indicated changes in neuronal gene expression in AD brain (e.g. 21–23) and in transgenic mouse models of AD (e.g. 24, 25). However, a central issue that remains to be determined is whether the changes in gene expression detected in those studies represent a late consequence of cerebral inflammation and neurodegeneration or a direct and early effect of AβOs on neuronal gene expression.

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5 The abbreviations used are: AD, Alzheimer disease; Aβ, amyloid-β; AβO, amyloid-β oligomer; qRT-PCR, quantitative reverse transcriptase PCR; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; GFAP, glial fibrillary acidic protein; LTD, long-term depression.
Here, we report an investigation of changes in gene expression induced by AβOs using human adult cortical slices as a novel experimental model to study the neuronal impact of AβOs. Using a combination of DNA microarray analysis and quantitative reverse transcriptase PCR (qRT-PCR), we found that sublethal concentrations of AβOs induce changes in expression of genes classified into several pathways important for neuronal physiology, including synaptophysin, a presynaptic vesicle membrane protein that has been implicated in synaptic failure in AD (26–28). A detailed understanding of the mechanisms by which AβOs affect neuronal gene expression and function may uncover early biomarkers of AD pathology and lead to the development of novel and effective therapeutics to prevent or halt the progression of AD.

EXPERIMENTAL PROCEDURES

Adult Human Cortical Slices—Adult human brain cortical fragments were obtained from patients with pharmacoresistant temporal lobe epilepsy submitted to hippocampectomy and anterior temporal lobectomy for removal of epileptic foci detected by magnetic resonance imaging (MRI). Only healthy cortical tissue removed from the area approached as surgical access to the target hippocampus was used for culture purposes. The health of cortical tissues was determined based on the structural/anatomical integrity revealed by MRI (supplemental Fig. 1), macroscopic appearance, and lack of correlation with epileptic activity in scalp video-EEG monitoring. Donors (six men and four women) were 34 ± 9 years old and gave written informed consent for use of brain tissue that would otherwise have been discarded. All procedures were approved and regulated by the National Committee for Research Ethics (CONEP) of the Brazilian Ministry of Health (Protocol 0069.0.197.000-05).

Tissue was placed immediately in transport medium (50% (v/v) Hanks’ balanced salt solution containing 10 mM HEPES, 3 mg/ml glucose, and 50 μg/ml gentamicin diluted in Neurobasal A medium supplemented previously with 2% B27 and 0.5 mM serum, and 1% Triton X-100 for 6 h at room temperature) in Neurobasal A/B27 (Invitrogen) medium with antibiotics. One-third of the medium was replaced after 3 days in culture.

Dissociated Hippocampal Neuronal Cultures—Hippocampal neuronal cultures were prepared from 18-day-old rat embryos as described previously (29, 30). Briefly, hippocampi were dissected in PBS-glucose and mechanically dissociated, and cells were plated onto poly-l-lysine-coated wells at a density of 1.5 × 10^6 cells/well (for 35-mm wells) in Neurobasal/B27 medium with antibiotics. All procedures were approved by and followed the guidelines of the Institutional Animal Care and Utilization Committee of the Federal University of Rio de Janeiro (Protocol IBQM 022). After 21 days at 37 °C in a 5% CO₂ atmosphere, cultures were treated with vehicle or AβOs and further incubated at 37 °C for 12 or 24 h.

AβO Preparation—AβOs were prepared in PBS (31) with minor modifications as described previously (32) using Aβ(1-42) peptide from Bachem (Torrance, CA). The preparation was centrifuged at 14,000 × g for 10 min at 4 °C to remove any insoluble aggregates, and the supernatant containing soluble AβOs was transferred to clean tubes and stored at 4 °C. Protein concentration was determined using the BCA assay (Pierce). Oligomer solutions were used within 24 h of preparation. Routine characterization of oligomer preparations was performed by size-exclusion chromatography and Western blot and, occasionally, by transmission electron microscopy (supplemental Fig. 2). Collectively, the results indicate that our preparation comprises soluble oligomeric species including dimers, trimers, tetramers, and higher molecular mass oligomers of ~50–180 kDa, ranging in diameter from ~1.5 to 3.5 nm.

Live/Dead Viability Assay—Cell viability in cultured slices from three different donors was determined using the Live/Dead assay (Invitrogen). At different days in vitro, slices were exposed to dyes (4 μM calcein and 2 μM ethidium homodimer) for 40 min, washed twice with ice-cold PBS, and cut into 12-μm sections for imaging on a Nikon Eclipse TE300 microscope. Live or dead neurons were identified by green calcein fluorescence or red ethidium fluorescence, respectively. Percentages of live neurons are expressed relative to the total number of cells (determined by DAPI staining) in each slice.

Immunohistochemistry—After 4 days in culture, brain slices were fixed in 4% paraformaldehyde and double stained for mature neurons (mouse anti-NeuN, Chemicon; 1:100) and astrocytes (rabbit anti-GFAP, Dako; 1:200). Alexa 488 and Alexa 594 conjugates were used as secondary antibodies (Molecular Probes; 1:200). DAPI (Sigma) was used to visualize nuclei. Antibody incubations were performed using free floating sections (treated previously with 0.1 M citrate buffer, pH 6, at 60 °C for 5 min and blocked with 5% BSA, 5% normal goat serum, and 1% Triton X-100 for 6 h at room temperature) in PBS containing 1% Triton X-100. Primary antibodies were diluted in blocking solution and incubated for 48 h at 4 °C, followed by incubation with Alexa-conjugated secondary antibodies for 24 h at 4 °C. Tissue autofluorescence was quenched by incubation with 0.06% potassium permanganate for 10 min at room temperature.

MTT Assay—Hippocampal slices were incubated for 24 h in the presence of AβOs at different concentrations, and cell viability was analyzed by the MTT reduction assay carried out as described previously (13). Briefly, slices were incubated with a 0.5 mg/ml solution of MTT at 37 °C for 4 h to allow reduction to formazan blue by metabolically active cells. Cells were then lysed, and formazan crystals were solubilized by incubation in 0.01 N HCl containing 10% SDS for 24 h under agitation at room temperature. Optical density at 570 nm was measured in a ThermoMax Microplate reader.

RNA Extraction and Amplification—Total RNA from hippocampal cultures was extracted with TRizol (Invitrogen) following the manufacturer’s instructions. One milliliter of TRizol was used to extract RNA from 1.5 × 10^6 cells. The purity and integrity of RNA preparations were checked by the 260/280 nm ratio.
absorbance ratio and by agarose gel electrophoresis. Only preparations with 260/280 nm ratios ≥ 1.8 and no signs of RNA degradation were used. mRNA from human cortical slices was obtained using an RNA amplification protocol. After treatment with AβOs, slices were washed twice with PBS and kept in RNA later (Ambion) at −20 °C until use. For total RNA extraction, tissue was ground using an electric homogenizer, and RNA was purified using an RNeasy mini kit (Qiagen). Total RNA was then used as a template in a two-round linear amplification, tissue was ground using an electric homogenizer, and RNA was purified using an RNeasy mini kit (Qiagen). Total RNA was then used as a template in a two-round linear amplification procedure based on T7-driven amplification as described previously (33). The quality of amplified RNAs was checked by agarose gel electrophoresis upon visualization with ethidium bromide (supplemental Fig. 3). All samples presented a smear between 300 and 700 bp and no signs of RNA degradation.

**Microarray Analysis**—Ten μg of amplified RNA from each sample (three different donors, each used for both control and AβO-treated conditions, corresponding to six paired samples in total) were used as template for cDNA synthesis. cDNA samples were labeled indirectly with Alexa Fluor® 555 or Alexa Fluor® 647 reactive dye (Invitrogen) and IMPROM II reverse transcriptase (Promega) in a reverse transcriptase reaction and purified as described (34). Hybridization reactions were performed in duplicate with dye swapping using a human universal 4,800 chip (35), with excellent agreement between duplicates. After washing, slides were scanned on a confocal laser scanner (ScanArray Express, PerkinElmer Life Sciences), and data were extracted with ScanArray Express software. Self-self hybridization was utilized to define cutoff limits for differential gene expression (36). The percentile used was 0.95.

A list of differentially expressed genes induced by AβO treatment was used to identify the affected biological pathways using the Gene Set Analysis Toolkit V2 (37). The gene list used as input for the Kegg pathway analysis comprised both up- and down-regulated genes present at the intersections between at least two samples. The parameters used were as follows: organism, Homo sapiens; ID type, gene_symbol; reference set, Entrez Gene; significance level, 0.05; statistics test, hypergeometric; multiple test correction, Benjamini and Hochberg; minimum genes per category, 2.

**Quantitative RT-PCR Assays**—Quantitative expression analysis of genes of interest was performed by quantitative real-time PCR on an Applied Biosystems 7500 real-time PCR system with the Power SYBR kit (Applied Biosystems). For human tissue, cDNA converted from amplified RNA was used as the template. This procedure has been found not to introduce any bias on relative gene expression measurement (38). Three reference human genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), hypoxanthine phosphoribosyltransferase 1 (HPRT), and β-actin (ACTB), were used to generate a single normalization factor as described (39).

For rat neuronal cultures, template cDNA was prepared from total RNA (1 μg) using 50 pmol of oligo dT160 and the Super-Script III First Strand cDNA kit (Invitrogen), and ACTB or GAPDH was used as the endogenous control. Cycle threshold (Ct) values were used to calculate -fold changes in gene expression using the 2−ΔΔCt method (40). In all cases, reactions were performed in 20-μl reaction volumes.

**Western Blotting**—Mature rat embryonic hippocampal cultures were treated with AβOs (500 nM) or vehicle for 12 or 24 h, rinsed with PBS, and lysed in buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1.5 mM MgCl2, 1.5 mM EDTA, 1% Triton X-100, 10% glycerol, and Halt™ protease inhibitors mixture (Thermo Fisher Scientific, Rockford, IL). Human brain slice extracts were prepared using the same buffer in the ratio of 50 μl/slice. The protein content in the extracts was determined using the BCA™ protein assay kit (Thermo Fisher Scientific). Extracts (100 μg of protein/lane) were resolved on 12% SDS-PAGE. Proteins were transferred to nitrocellulose membranes (Hybond™-C Extra, Amersham Biosciences) for 90 min at 100 V. Membranes were blocked with 3% BSA in Tris-buffered saline/Tween 20 (TBS-T: 10 mM Tris, pH 7.2, 150 mM NaCl, and 0.1% Tween 20), followed by overnight incubation with anti-synaptophysin monoclonal antibody (5 μg/ml; Sigma-Aldrich) and polyclonal anti-cyclophilin B (0.01 μg/ml; Abcam) at 4 °C. After washing with TBS-T, immunoreactivity was visualized using peroxidase-conjugated anti-rabbit IgG secondary antibody (1:10,000 dilution; Zymed Laboratories Inc., Carlsbad, CA) for cyclophilin B detection (used as a loading control) and anti-mouse IgG secondary antibody (1:50,000 dilution; Amersham Biosciences) for synaptophysin detection. SuperSignal® West Pico chemiluminescent detection (Thermo Fisher Scientific) was used for visualization. Densitometric scanning and quantification were carried out using NIH ImageJ (Windows version).

**RESULTS**

**Exposure to a Sublethal AβO Concentration Alters Gene Expression in Human Cortical Slices**—Organotypic cultures of post-mortem human cortex have been previously prepared (41). Here, starting from ex vivo human cortical tissue, we developed a novel experimental model to investigate the neuronal impact of Aβ oligomers. Slices from healthy cortical tissue obtained from adults submitted to surgical removal of hippocampal epileptic foci were maintained successfully in vitro for up to 25 days with cell viability greater than 50% (Fig. 1). Semiquantitative analysis of cell types in slices from three different donors revealed an average of 60% neurons and 21% GFAP-positive cells in cortical human slices cultured for 4 days. Previous studies have shown that exposure of dissociated neuronal cultures (for up to 24 h) to AβOs at submicromolar concentrations causes neuronal dysfunction in the absence of cell death (8, 17) (reviewed in Ref. 5). Consistent with those findings, no change in cell viability was detected in human cortical slices exposed to AβOs (500 nM) for 24 h compared with control, vehicle-treated slices (supplemental Fig. 4).

Following treatment with AβO, mRNA was extracted from slices and hybridization was performed on a customized cDNA platform containing 4608 human genes (35). Microarray analysis revealed significant alterations in gene expression induced by AβOs compared with paired vehicle-treated slices from the same donors (Fig. 2). Considering all of the genes identified as up- or down-regulated that were present in at least two independent donors, a total of 345 genes were found to be differentially expressed upon AβO treatment (72% of which were down-regulated (Fig. 2A)), corresponding to ~7% of the total number of genes represented on the chip. When the intersec-
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FIGURE 1. Cell viability in cultured slices from ex vivo adult human cortex. After 25 days in vitro, slices were incubated with calcein and ethidium homodimer and imaged. A–C, representative images of a slice. A, bright field. Scale bar: 400 nm. B, ethidium homodimer + DAPI merged image. C, calcein + ethidium homodimer merged image. Dead cells can be readily identified among total (DAPI-stained) cells by red ethidium homodimer staining (B), in contrast with green calcein fluorescence emitted by live cells (C). D, cell viability in slices obtained from a single donor. Viability averaged 62 ± 1.4% for this particular experiment. E, no significant reduction in viability was noted after several days in culture using slices prepared from three different donors. F, immunohistochemistry of slices after 4 days in culture showing neurons (green, labeled using neuronal marker NeuN) and astroglial cells (red, labeled using anti-GFAP antibody). Scale bar: 40 nm. Semiquantitative analysis of cell types in slices from three different donors revealed an average of 60% neurons and 21% GFAP-positive cells in cortical human slices cultured for 4 days.

Functional Classification of Differentially Expressed Genes in AβO-treated Slices—To assign biological functions to the list of AβO-induced differentially expressed genes, we mapped both up- and down-regulated genes present in hybridizations from at least two donors to specific biological Kegg pathways (42), an approach known to add confidence to the results found for expression of individual genes (43). This analysis revealed 18 pathways that were significantly over-represented (p = 0.05) in AβO-treated tissue (Fig. 3 and supplemental Table 3). Significantly, six of those pathways (“SNARE interaction in vesicular transport,” “Alzheimer’s disease,” “axon guidance,” “long-term depression,” “regulation of actin cytoskeleton,” and “focal adhesion”) have been implicated in the mechanisms of pathogenesis in the central nervous system. Of considerable interest was finding the “insulin-signaling pathway” among the over-represented pathways, as impaired neuronal insulin signaling has been linked recently to AD pathogenesis and, specifically, to the impact of AβOs (18, 19). Another over-represented pathway previously associated with energy metabolism and AD pathogenesis is the “adipocytokine-signaling pathway”, as leptin, an adipocytokine involved in fatty acid metabolism, has recently been reported to control Aβ levels (44). It is noteworthy that, among the 41 distinct genes mapped to the 18 over-represented pathways (representing 12% of the total number of differentially expressed genes), 63% were down-regulated by AβOs. Functional classification analysis thus suggests that AβOs down-regulate expression of genes crucial for proper neuronal function and plasticity, establishing organotypic cultures of adult human brain cortex as a novel experimental model that effectively recapitulates the association between molecular abnormalities and clinical manifestations of AD.

Validation of AβO-induced Changes in Gene Expression—Selected differentially expressed genes identified by microarray analysis were validated by qRT-PCR using the same set of cortical slice samples plus four extra pairs of AβO- and vehicle-treated samples obtained from independent donors not included in the microarray analysis. The basic criterion used to select genes for qRT-PCR analysis was that they should be present at the intersection between the three donors used in the microarray analysis (Fig. 2C and supplemental Tables 1 and 2). mRNA levels of 4 of 6 up-regulated annotated genes and 11 of 17 down-regulated annotated genes were determined directly by qRT-PCR (Fig. 4), corresponding to 65% of the differentially expressed genes identified at the intersection among three donors in the microarray data (Fig. 2C). Two additional genes tested (rbed and prlr) rendered inconclusive results because of very low basal expression levels. Primer sequences for all of the genes can be found in supplemental Table 4. Differential expression was confirmed by qRT-PCR for five down-regulated genes and one up-regulated gene (Fig. 4).

Synaptophysin Down-regulation at mRNA and Protein Levels in AβO-treated Hippocampal Neuronal Cultures and Human Brain Slices—Because synaptophysin has been implicated in synapse loss in AD (26, 45, 46) and its expression was markedly
down-regulated by AβOs (~50% decrease compared with vehicle-treated slices (Fig. 4)), we further investigated the effect of short-term exposure to AβOs (500 nM) on synaptophysin levels using mature rat hippocampal neuronal cultures. Consistent with the finding in human brain slices, exposure of neuronal cultures to AβOs for 12 h caused a significant reduction in synaptophysin mRNA level (Fig. 5A). Although synaptophysin protein levels were not affected by AβOs at 12 h of exposure, a longer (24 h) incubation caused a significant reduction in synaptophysin (Fig. 5B), indicating that AβO-induced down-regulation of synaptophysin mRNA is followed by a comparable reduction in the protein level. Importantly, corroborating our microarray/qPCR findings, we also detected down-regulation of synaptophysin protein levels in AβO-treated human brain slices obtained from three new independent donors (Fig. 5C).

DISCUSSION

Changes in gene expression have been reported as part of AD pathology, but a general consensus is still lacking as to which genes or cellular pathways are primarily affected, especially at early stages of the disease. Previous studies have relied largely on the analysis of post-mortem material, making it difficult to distinguish whether changes in transcription were elicited directly by AβO toxic signaling or were a consequence of events taking place at later stages of the disease, including brain
inflammation and neurodegeneration. In the present study, we present the first demonstration that AβOs (used at a sublethal concentration) markedly affect gene expression in the absence of overt neurodegeneration in adult human brain slices in culture.

Analyses of changes in gene expression in post-mortem AD brain have yielded controversial results. For example, up-regulation of tumor suppressor genes (21) and down-regulation of retromer trafficking complex genes (22) have each been reported independently as the most significant transcriptional alteration in AD hippocampus. Similarly, in cortical tissue from AD patients, expression of both immunity-related MHC II (47) and calcium-signaling genes (48) were found to be dysregulated. More recently, an elegant study employing cultivated slices from post-mortem AD brain (23) described significant alterations in the expression of genes related to synaptic activity and β-amyloid processing in prefrontal cortex in the presymptomatic stage, when plaque pathology was not detected. In addition to post-mortem AD brain, transgenic mouse models of AD have been used to detect gene expression alterations relevant to AD pathogenesis. Again, there is no consensus among transcriptional alterations reported in those studies. For example, Reddy et al. (25) found up-regulation of genes involved in mitochondrial energy metabolism in animals expressing human APP harboring an AD-associated mutation (Tg2576 mice), whereas the down-regulation of genes important for memory consolidation was reported in a study using a double transgenic mouse model (APP + PS1 human transgenes) (24). In addition to technical considerations (e.g. different mouse models or DNA microarray platforms employed), it is likely that differences between specific molecular and cellular events underlying AD pathogenesis and findings in animal models or in post-mortem tissue contributed to the differences in outcome in the studies mentioned above.

Animal models do not mirror the full range of AD symptoms (49, 50), and studies with post-mortem human brain samples are often hampered by difficulties in determining the effect of factors such as the post-mortem interval, brain pH, age, disease status, and other neurological conditions affecting tissue quality (51, 52). As a novel approach to gain insight into clinically relevant disease mechanisms that cannot be properly addressed using post-mortem or animal model brain tissue, here we introduced the use of cultured adult human brain slices as a model to investigate AβO-induced AD pathology. To date, a single study has investigated the effects of AβO treatment on transcription (53) and revealed down-regulation of genes involved in protein modification and degradation. However, the possible physiopathological significance of those findings appears questionable, as the concentration of AβOs used (50 μM) was orders of magnitude higher than the concentrations found in the diseased human brain. Moreover, that study made use of an immortalized cell line (rather than neurons), further complicating correlation of the findings with human brain neuropathology.

The present work constitutes the first description of the early effects of AβOs at low concentrations on gene expression in adult human neurons under conditions in which cell viability is not impaired. The investigation of the impact of AβOs on human brain tissue may provide insight into mechanisms that are directly relevant to AD pathogenesis but might not be apparent in studies based on transgenic rodent models or dissociated cell cultures.

Quantitative RT-PCR was used to validate microarray results. The percentage of validation of differential expression was higher in the set of down-regulated genes, consistent with the finding that AβOs predominantly induced down-regulation rather than up-regulation of gene expression (Fig. 2A). Moreover, down-regulation of synaptophysin (SYP) and vaccinia-related kinase 3 (VRK3), two of the three genes with the highest level of down-regulation in the microarray analysis (Fig. 2C), was validated by qPCR (Fig. 4), confirming that the most significant alterations detected in the microarray analysis indeed reflect gene expression changes triggered by AβO treatment. qPCR data also validated the up-regulation of cytoplasmic FMR1-interacting protein 1 (CYFIP1), initially detected in the microarray analysis (Fig. 2C). CYFIP1, along with EIF4E, forms a complex with Fragile X mental retardation protein (FMR1), a key regulator of translation in dendritic spines known to be down-regulated in the Fragile X syndrome (54). It has been proposed recently that dementia in AD and mental...
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retardation in Fragile X syndrome share common pathogenic features and may both be consequences of synaptic dysfunction caused by loss of or decreased FMR1 activity (6, 55). Therefore, it is possible that AβO-induced up-regulation of CYFIP1 is related to disruption of FMR1-mediated control of translation in AD.

Synaptophysin loss has been extensively associated with AD pathology. Synaptophysin mRNA and protein levels are reduced in the post-mortem AD brain (45, 46), and protein levels are also found to be down-regulated in animal model brains (56, 57). Synaptophysin was the most significantly down-regulated gene in our analysis, establishing a clear correlation between our findings and a typical AD-type pathology. Interestingly, whereas previous studies have investigated synaptophysin as a marker of AD pathology rather than a direct target of AβOs, we now show that significant reductions in synaptophysin mRNA and protein levels are induced by short-term AβO treatment (Fig. 5). These results implicate synaptophysin down-regulation as an early abnormality triggered by AβO toxic signaling and synapse loss.

Synaptophysin plays an important role in intraneuronal vesicle transport, namely in the processes of presynaptic vesicle membrane fusion and neurotransmitter release in the synaptic cleft (58). Interestingly, current results indicated that “SNARE interactions in vesicle transport” was the most significantly overrepresented pathway in AβO-treated slices (supplemental Table 3). Two additional important SNARE proteins in which gene expression was affected by AβOs are VAMP and syntaxin (Fig. 3), both involved in presynaptic vesicle fusion to the plasma membrane and neurotransmitter release (59). Along with previous reports of AD-associated alterations in neuronal vesicle transport machinery at the mRNA level (22), current data indicate that AβOs promote a rapid and specific attack on the neuronal vesicle transport system, which likely culminates in synaptic dysfunction.

Increasing evidence supports a recently proposed link between human brain insulin resistance and AD pathology (18, 60–65). At the cellular level, AβOs have been shown to induce insulin receptor internalization and the impairment of neuronal insulin signaling in hippocampal cultures (18, 19). In line with those studies, we found here that the insulin-signaling pathway is one of the pathways targeted by AβOs (Fig. 3). Interestingly, among the genes identified in the present study are two key components of insulin signaling, AMP-activated kinase (AMPK) and mTOR (Fig. 3, gene symbols prkag1 and frap1, respectively). The possible involvement of mTOR in the pathophysiology of AD is still a controversial matter. For example, Spilman et al. (66) show that pharmacological inhibition of mTOR by rapamycin decreases Aβ42 levels and rescues the cognitive function in a transgenic mouse model of AD, suggesting that mTOR activity may be increased in Aβ-related disease. Conversely, Ma et al. (67), also using AD transgenic mice (albeit a different strain from that used by Spilman et al. (66)), found that mTOR signaling was inhibited both in cultured neurons and hippocampal slices from AD-transgenic mice and in wild-type hippocampal slices exposed to exogenous Aβ1–42 and that this mTOR dysregulation correlated with impairment in synaptic plasticity. It is thus possible that different genetic backgrounds may lead to distinct responses in terms of mTOR activity in transgenic mice. The present observation of alterations in the expression of mTOR and other proteins involved in insulin signaling in AβO-treated human tissue may reflect an early pathological response not apparent in transgenic mice (which exhibit exacerbated amyloid pathology) or AD post-mortem samples. Taken together, the current results, along with recent and discrepant literature reports, warrant further studies to elucidate the role of mTOR and functionally associated proteins in AD-linked insulin resistance, particularly in the early stages of the disease. In addition, the results suggest that transcriptional dysregulation of genes responsive to insulin signaling may be part of the mechanism by which neuronal insulin resistance is induced by AβOs.

Ubiquitin-mediated proteolysis, a pathway found to be altered by AβO treatment (Fig. 3), has also been described as part of AD pathogenesis (22, 68). One of the genes grouped in this pathway is cullin 3, which encodes an essential component of the ubiquitin E3 ligase complex. We found a significant down-regulation of cullin 3 in AβO-treated slices, in accord with results reported using post-mortem AD hippocampal tissue (21). Down-regulation of the ubiquitin system at the protein level in AD has also been reported previously (69), strengthening the possibility that ubiquitin-mediated systems are specific targets of AβO toxicity.

Cell adhesion molecules are well known players in the regulation of synaptic plasticity, learning, and memory (70) and have been suggested to be involved in Aβ-mediated neurotoxicity (71, 72). We found that the cell adhesion-related pathways “cell adhesion molecules” and “focal adhesion”, as well as “regulation of actin cytoskeleton” (a crucial intracellular component of the cell adhesion machinery), were among the AβO-targeted pathways in human slices (Fig. 3). Furthermore, expression of the acting-binding protein CYFIP1, a component of the complex that regulates cytoskeleton remodeling in neurons (73), was up-regulated in AβO-treated slices from five of the seven donors tested by qPCR (Fig. 4). These results are consistent with the notion that alterations in cell adhesion are involved in synaptic failure in AD.

Aβ has been shown to impede the reversal of long-term depression (LTD) in rat hippocampal slices (74, 75). Here we found that AβOs disrupt the expression of genes associated with LTD (Fig. 3). Facilitation of LTD has been proposed as a mechanism by which AβOs cause synapse failure in AD (20, 76), and it is possible that altered expression of genes involved in LTD may underlie the dysfunctional synaptic plasticity instigated by AβOs.

In conclusion, we found that short-term exposure to a sub-lethal concentration of pathologically relevant AβOs disrupts gene expression in adult human brain slices, causing, in most cases, the down-regulation of genes important for neuronal physiology. Changes in gene expression found in the present study shed light on mechanisms by which AβOs affect neuronal gene expression and function and may be considered early events in AD pathology and, therefore, potential targets for the development of novel diagnostic or therapeutic approaches in AD.
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