Chronic Overexpression of the Calcineurin Inhibitory Gene DSCRI (Adapt78) Is Associated with Alzheimer’s Disease*

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The DSCRI (Adapt78) gene was independently discovered as a resident of the “Down syndrome candidate region” and as an “adaptive response” shock or stress gene that is transiently induced during oxidative stress. Recently the DSCRI (Adapt78) gene product was discovered to be an inhibitor of the serine/threonine phosphatase, calcineurin, and its signaling pathways. We hypothesized that DSCRI (Adapt78) might also be involved in the development of Alzheimer’s disease. To address this question we first studied DSCRI (Adapt78) in multiple human tissues and found significant expression in brain, spinal cord, kidney, liver, mammary gland, skeletal muscle, and heart. Within the brain DSCRI (Adapt78) is predominantly expressed in neurons within the cerebral cortex, hippocampus, substantia nigra, thalamus, and medulla oblongata. When we compared DSCRI (Adapt78) mRNA expression in post-mortem brain samples from Alzheimer’s disease patients and individuals who had died with no Alzheimer’s diagnosis, we found that DSCRI (Adapt78) mRNA levels were about twice as high in age-matched Alzheimer’s patients as in controls. DSCRI (Adapt78) mRNA levels were actually three times higher in patients with extensive neurofibrillary tangles (a hallmark of Alzheimer’s disease) than in controls. In comparison, post-mortem brain samples from Down syndrome patients (who suffer Alzheimer’s symptoms) also exhibited DSCRI (Adapt78) mRNA levels two to three times higher than controls. Using a cell culture model we discovered that the amyloid β_{1-42} peptide, which is a major component of senile plaques in Alzheimer’s, can directly induce increased expression of DSCRI (Adapt78). Our findings associate DSCRI (Adapt78) with such major hallmarks of Alzheimer’s disease as amyloid protein, senile plaques, and neurofibrillary tangles.

Alzheimer’s disease is characterized by specific neuronal degeneration in certain areas of the brain. The fundamental phenomena associated with this neurodegeneration are the development of senile plaques and neurofibrillary tangles. Plaques represent extracellular deposits of amyloid peptides, and tangles are formed by accumulation of tau and other proteins within neurons. Mutations and abnormal expression of several genes is associated with β-amyloid deposits and Alzheimer’s disease; among them APP (1), PS1 (2), and PS2 (3). It was also demonstrated that different alleles of the APOE gene can regulate susceptibility to Alzheimer’s disease (4, 5). Despite these findings, the mechanism of plaque development is not fully understood, and the role of amyloid deposition in Alzheimer’s disease is still debated (6–8). Even less is known about the development of neurofibrillary tangles and their exact role(s) in the disease.

There is growing evidence that oxidative damage is involved in the progression of Alzheimer’s disease (see Refs. 7, 9, and 10), and investigation of pathways associated with oxidative stress might be important in understanding the etiology of this disease. Elevated levels of oxidized proteins and lipids, and elevated endogenous antioxidant enzyme levels, have been observed in post-mortem brains from Alzheimer’s patients (11–14), and protein carbonyls and malondialdehyde are both associated with senile plaques and neurofibrillary tangles (15, 16). Because the brain represents only about 2% of human body weight but consumes about 20% of all the oxygen we breathe, one might expect high oxidation levels in brain cells and concomitantly effective mechanisms of protection against oxidative stress. One of the candidate antioxidant systems in brain might be encoded by the DSCRI (Adapt78) gene.

Adapt78 was originally discovered as an oxidant stress-inducible gene in hamster fibroblasts where it was suggested to serve a protective function against oxidative damage (17–19). Despite some initial sequence anomalies, it soon became clear that Adapt78 and DSCRI are, in fact, one and the same gene (17–21). DSCRI (Adapt78) is localized in the Down syndrome candidate region of chromosome 21 (20, 21). Alzheimer’s disease commonly develops in the fourth or fifth decade of Down syndrome, and it is likely that these two diseases share some common pathways (22). In addition, many familial cases of Alzheimer’s disease have been linked to chromosome 21 (23).

Recently it has been demonstrated that DSCRI interacts physically and functionally with the catalytic domain of calcineurin (24, 25). Calcineurin is a calcium/calmodulin-dependent serine/threonine phosphatase that has been shown to be involved in apoptosis of neuronal cells (26–28), which might be a key factor in Alzheimer’s disease (29), and to play a role in learning and memory processes (30). These two phenomena relate calcineurin with Alzheimer’s disease, and it is possible that DSCRI (Adapt78) might antagonize Alzheimer’s disease by regulation of calcineurin. On the other hand, it has recently been shown that calcineurin activity is decreased in Alzheimer’s disease (31), which, we postulated, could be caused by overexpression of DSCRI (Adapt78). Diminished calcineurin activity has been demonstrated to permit hyperphosphorylation of tau (28) and the consequent formation of neurofibrillary tangles that we associate with Alzheimer’s disease.

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has been demonstrated as a major enzyme in Ca\textsuperscript{2+}-dependent eukaryotic signal transduction pathways (32), and it is interesting that \textit{Adapt78} induction during adaptation to oxidative stress was shown to be a calcium-dependent process (17, 18).

We initiated the present studies to determine whether \textit{DSCR1 (Adapt78)} is overexpressed in Alzheimer's disease or is associated with neurofibrillary tangles or \(\beta\)-amyloid accumulation. To properly address these questions we had first to determine the pattern of \textit{DSCR1 (Adapt78)} expression in various brain regions and by different cell types (neurons versus astrocytes and microglia) and to test for possible age- or sex-related differences in gene expression. We also tested whether exposing cells directly to \(\beta\)-amyloid could induce \textit{DSCR1 (Adapt78)} expression.

**EXPERIMENTAL PROCEDURES**

**Post-mortem Human Brain Tissue**

Brain tissue used in this project was provided by the Alzheimer's Disease Research Center, University of Southern California and the Institute for Brain Aging and Dementia, University of California, Irvine, CA. Samples from the hippocampus, cerebral cortex, and cerebellum with post-mortem intervals of 3–12 h were fixed at \(-70^\circ\text{C}\) until use. The brains were examined according to the Consortium to Establish a Registry for Alzheimer's Disease. Every sample was accompanied by a full medical history and by detailed neuropathology summaries that included microscopic exams. Standard silver (Bielschowsky) and thioflavins stains were used to detect amyloid plaques and neurofibrillary tangles. All Alzheimer's disease patients met the NINDS-ADRDA criteria.

**Rat Brain Tissue**

Five- and twenty-four-month-old F344 × BN-F1 male rats were used in our experiments. The animals were fed \textit{ad libitum}. Brain tissue was dissected immediately after the rats were killed and stored at \(-70^\circ\text{C}\) until used.

**RNA Isolation**

Total RNA was extracted using the TRIzol reagent (Life Technologies, Inc.) following the manufacturer's protocol. Integrity of the RNA was estimated following agarose gel electrophoresis, and only RNA samples displaying discreet 28 S and 18 S RNA bands were used in our experiments. RNA concentrations were quantified spectrophotometrically, and relative content was further confirmed on ethidium bromide-stained gels.

**Northern Hybridization**

Samples containing 30 \(\mu\)g of human RNA and 10 \(\mu\)g of rat total RNA were subjected to electrophoresis through 1% agarose formaldehyde gels, blotted to nylon membranes (Oncor) with high efficiency transfer solution (Cinna/Biotec Laboratories) and cross-linked by ultraviolet radiation. The membranes were prehybridized for 4 h and hybridized for 15 h in Hybrizol I (Oncor) at 42 °C. After hybridization they were washed with 2 × SSC plus 0.1% SDS at room temperature for 1 and 10 min, then with 0.1 × SSC plus 0.1% SDS at 60 °C for 10 and 30 min. The membranes were exposed, developed and scanned using the PhosphorImager system (Molecular Dynamics).

To rehybridize RNA blots, hybridized and labeled probes were removed by washing the membranes in a solution of 0.1 × SSC, 0.1% SDS, and 10 mM Tris-HCl (pH 7.0) at 90 °C for 10 min. To quantify the level of \textit{DSCR1 (Adapt78)} mRNA expression the membranes were scanned and the hybridization signal measured using ImageQuant software (Molecular Dynamics). Each signal was recalculated according to the amount of RNA actually loaded on the gels. The amount of the loaded RNA was controlled using hybridization with a GAPDH\textsuperscript{1} probe. Probes containing \([\alpha-\text{32P}]\text{dCTP}\)-labeled DNA were prepared using the High Prime system (Roche Molecular Biochemicals). A fully cloned hamster \textit{Adapt78} fragment (17) was used to prepare \textit{DSCR1 (Adapt78)} probes, and a PCR fragment consisting of exons 7 and 8 was used to prepare GAPDH probes (see below).

1 The abbreviations used are: GAPDH, glyceraldehyde-3-phosphate dehydrogenase gene; PCR, polymerase chain reaction; PBS, phosphate-buffered saline.

Sections were air-dried and fixed in fresh buffered 4% paraformaldehyde for 30 min. They were then rinsed in phosphate-buffered saline (PBS) for 5 min (two to three times) and blocked for endogenous peroxidases by incubating for 20 min in a solution containing 10% methanol and 0.3% hydrogen peroxide in PBS. Sections were rinsed in PBS for 5 min (three times), incubated in 1% Nonidet P-40 (in PBS) for 5 min, and then washed twice in PBS. Tissues were placed in blocking solution consisting of 1% normal rabbit sera in 10 ml of fresh stock solution for 30 min. Stock solution was PBS containing 0.3% Triton X-100, 1% heparin, 1 mM diithiothreitol, and 100 units/ml RNase. Sections were incubated for 90 min in primary antibody solution. The primary antibody solutions were prepared by dilution of the cell-specific primary antibodies in stock solution: anti-neuronal nuclei monoclonal antibody (NeuN,Chemicon International Inc.) and antibodies to neuron-specific enolase were diluted to 1:500; anti-glial fibrillary acidic protein solution (Roche Molecular Biochemicals) for detection of astrocytes was diluted 1:30; anti-HLA-DR (Dako) to detect human microglial cells was diluted 1:500; and anti-CD11b ox-42 (Serotec Ltd.) to detect rat microglial cells was diluted 1:500.

Sections were rinsed in PBS containing 0.1% Tween 20 for 5 min (three times) and incubated with rabbit-preabsorbed anti-mouse IgG for 60 min. The Vectastain ABC Kit (Vector Laboratories) was used for subsequent steps using diaminobenzidine as the substrate, following the manufacturer's protocol. Finally, slides were rinsed in PBS and processed for in situ hybridization as described below.

**In Situ Hybridization**

Brain sections were treated with acetic anhydride in 0.1 M triethanolamine, rinsed in PBS, dehydrated, and hybridized with antisense or sense (negative control) \(^{35}\text{S}\)-labeled RNA probe.

**RNA Probe Synthesis—** \textit{A DSCR1 (Adapt78)} fragment spanning exons 1, 5, 6, and 7 was synthesized by RT-PCR as described below. The fragment was cloned into the Smal site of the pBluscript II SK vector, which is located between the recognition sites for T3 and T7 polymerases; this allowed us to synthesize both sense and antisense RNA probes. To ensure that the correct sequences had been cloned, and to determine their orientation in each vector, all fragments were then sequenced.

**RT-PCR**

The synthesis of first-strand cDNA was performed using the SuperScript preamplification system from Life Technologies, Inc. 1–3 \(\mu\)g of total RNA from human brain per reaction was reverse-transcribed using oligo(dT) as the primer. About 2 \(\mu\)l out of 20 \(\mu\)l of the total volume
of the cDNA was used per each PCR reaction. Primers to amplify human DSCR1 (Adapt78) mRNA isoform consisting of exons 1, 5, 6, and 7 were as follows: 5'-GACTGGAGCTTCATTGACTGCGAGA-3' and 5'-ACCACGGTGGAGTGGTGTGGTGTCAGTCG-3', corresponding to bases 1–25 of exon 1, and 5'-ACCACGGTGGAGTGGTGTGGTGTCAGTCG-3', corresponding to bases 1–25 of exon 7.

The correct sequence of the fragment was verified by sequencing using an ABI Prism 377 DNA sequencer (PerkinElmer Life Sciences) in our core facility.

**RESULTS**

**High Levels of DSCR1 (Adapt78) mRNA in Human Brain**—We assessed DSCR1 (Adapt78) mRNA levels in human tissues using an RNA master blot (CLONTECH Laboratories Inc.). The RNA master blot containing poly(A)+ RNA from 50 different human tissues was first hybridized with DSCR1 (Adapt78) probe (Fig. 1) and then with GAPDH to control the loading (not shown). The same blots (each carrying exactly the same amount of each RNA) were also probed with radioactively labeled ubiquitin control cDNA probe and a major histocompatibility complex class Ic control cDNA. The presence of consistent signals was confirmed for all 50 poly(A)+ RNA dots using the ubiquitin probe, and the major histocompatibility complex probe produced the expected expression pattern (not shown). As seen in Fig. 1, DSCR1 (Adapt78) is widely expressed in different human tissues. Significant steady-state levels of DSCR1 (Adapt78) mRNA were detected in brain, spinal cord, kidney, liver, mammary gland, placenta, skeletal muscle, and heart. The brain regions, which expressed DSCR1 (Adapt78) mRNA most strongly, were cerebral cortex, hippocampus, substantia nigra, thalamus, and medulla oblongata.

**Brain DSCR1 (Adapt78) mRNA Expression Is Not Significantly Affected by Aging or Gender**—Alzheimer's disease predominantly (although not exclusively) affects older individuals. It was, therefore, necessary to determine whether DSCR1 (Adapt78) expression is affected solely by age. We examined post-mortem brain samples from 16 patients aged between 48 and 92 years old (Table I). Samples from four different brain areas were studied for each of the 16 patients: cerebral cortex, hippocampus, and two different areas of cerebral cortex (A10, which is frontal, and A22, which is the temporal region according to Brodmann's map (33)). DSCR1 (Adapt78) mRNA levels in these samples were assessed using Northern hybridization. Total RNA samples were separated on agarose gels, blotted to membranes, hybridized to a DSCR1 (Adapt78) probe, and compared. The hybridization signal was quantified using ImageQuant software (Molecular Dynamics) and expressed relative to the GAPDH loading probe signal. The results revealed no correlation between patient age and DSCR1 (Adapt78) mRNA levels in the four brain areas studied (Fig. 2A).

We also analyzed brain samples from rats and obtained similar results. The F344 × BN-F1 rat hybrid line, which is known as "successful agers" (34) was used in this study. The typical life span of this rat line is about 3 years, and at the age of 24 months they remain healthy. We examined expression of DSCR1 (Adapt78) mRNA in the cerebellum and cerebral cortex of nine young (5 months old) and nine older (24 months old) rats by Northern hybridization. The procedure used was essentially the same as that described above for analysis of human samples. The results showed no significant difference in DSCR1 (Adapt78) mRNA levels between young and older rats in either the cerebellum or the cerebral cortex (Fig. 2B).

Finally, we re-analyzed our human data, comparing men and women in age- and disease-matched samples. We found no significant differences between men and women in DSCR1 (Adapt78) expression in the A10 or A22 areas of cerebral cortex, in the hippocampus, or in the cerebellum (data not shown).

**Expression of the DSCR1 (Adapt78) Gene Is Up-regulated in Human Brains Affected by Alzheimer's Disease**—Alzheimer's disease does not affect the cerebellum, and it was expected that the cerebellar level of DSCR1 (Adapt78) expression would also be unaffected by the disease. Since cerebellar levels of DSCR1 (Adapt78) could provide a very important control for comparing Alzheimer's patients and non-Alzheimer's individuals, this possibility was assessed using Northern hybridization. Post-mortem RNA samples were isolated from cerebells of non-Alzheimer's patients and compared with age-matched samples from seven patients diagnosed with Alzheimer's disease. None of these samples had any kind of abnormalities, including amyloid plaques or neurofibrillary tangles. Samples of total RNA were separated on the same gels and directly compared. The hybridization signal was estimated as described in the legend to Fig. 2A. The analysis revealed no significant differences in DSCR1 (Adapt78) mRNA expression between these two groups.

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**Table:**

| Brain Region | Sample Type |
|--------------|-------------|
| Cerebral Cortex | |
and confirmed that the expression of DSCR1 (Adapt78) in cerebellum is not affected by Alzheimer’s disease (also see data from more samples in Fig. 2).

We next measured the level of DSCR1 (Adapt78) mRNA production in different areas of each brain, relative to those in the cerebellum, which was used as internal control for each patient. RNA samples isolated from various brain regions from eight patients who had died with Alzheimer’s disease were compared with samples isolated from the corresponding brain regions from eight patients who had died with no signs of Alzheimer’s disease. The ages of the patients were matched, averaging 78 years for the Alzheimer’s disease group of patients and 71 for the control group. We compared the levels of DSCR1 (Adapt78) mRNA production in brain areas that generally are affected by Alzheimer’s disease: hippocampus and two different areas of cerebral cortex (A10, which is frontal, and A22, which is temporal region according to Brodmann’s map).

Only RNA samples that displayed discreet 18 S and 28 S rRNA bands with no degradation were used in our studies. In addition, as shown by the examples in Fig. 3, only samples displaying discreet DSCR1 (Adapt78) hybridization bands, and GAPDH hybridization bands, were used (Fig. 3). These precautions proved the integrity of the samples used in this study. All samples from Alzheimer’s disease patients showed abundant neurofibrillary tangles and amyloid plaques that included mature neuritic plaques. The samples from control patients had no amyloid plaques or neurofibrillary tangles, except samples from patient number 9, which had some neurofibrillary tangles (Table I). Northern hybridization analysis revealed that the level of DSCR1 (Adapt78) expression in cerebral cortex and

| Patient number | Age (years) | Sex | Final diagnosis and notes          |
|----------------|-------------|-----|-----------------------------------|
| Alzheimer’s disease cases |             |     |                                    |
| 1              | 87          | Female | Alzheimer’s disease.              |
| 2              | 92          | Female | Alzheimer’s disease.              |
| 3              | 80          | Male   | Alzheimer’s disease.              |
| 4              | 48          | Female | Alzheimer’s disease.              |
| 5              | 84          | Female | Alzheimer’s disease.              |
| 6              | 84          | Male   | Alzheimer’s disease.              |
| 7              | 77          | Female | Alzheimer’s disease.              |
| 8              | 73          | Male   | Alzheimer’s disease.              |
| Control cases  |             |     |                                    |
| 9              | 80          | Male   | Pneumonia. Neurofibrillary tangles in hippocampus and cortex. |
| 10             | 71          | Female | Chronic obstructive pulmonary disease. |
| 11             | 88          | Male   | Subdural hematoma.                |
| 12             | 68          | Female | Bladder cancer.                   |
| 13             | 70          | Male   | Amyotrophic lateral sclerosis.    |
| 14             | 54          | Female | Amyotrophic lateral sclerosis.    |
| 15             | 70          | Male   | Cardiac arrest.                   |
| 16             | 65          | Male   | Cardiac arrest.                   |

**Table I**

Details of the Alzheimer’s disease and control cases

| Patient number | Age (years) | Sex | Final diagnosis and notes          |
|----------------|-------------|-----|-----------------------------------|
| 1              | 87          | Female | Alzheimer’s disease.              |
| 2              | 92          | Female | Alzheimer’s disease.              |
| 3              | 80          | Male   | Alzheimer’s disease.              |
| 4              | 48          | Female | Alzheimer’s disease.              |
| 5              | 84          | Female | Alzheimer’s disease.              |
| 6              | 84          | Male   | Alzheimer’s disease.              |
| 7              | 77          | Female | Alzheimer’s disease.              |
| 8              | 73          | Male   | Alzheimer’s disease.              |
| 9              | 80          | Male   | Pneumonia. Neurofibrillary tangles in hippocampus and cortex. |
| 10             | 71          | Female | Chronic obstructive pulmonary disease. |
| 11             | 88          | Male   | Subdural hematoma.                |
| 12             | 68          | Female | Bladder cancer.                   |
| 13             | 70          | Male   | Amyotrophic lateral sclerosis.    |
| 14             | 54          | Female | Amyotrophic lateral sclerosis.    |
| 15             | 70          | Male   | Cardiac arrest.                   |
| 16             | 65          | Male   | Cardiac arrest.                   |

**Fig. 2.** Brain DSCR1 (Adapt78) mRNA expression during aging, in Alzheimer’s disease, and with neurofibrillary tangles. A, levels of DSCR1 (Adapt78) mRNA in brain autopsy samples from 16 individuals of different ages but with no diagnosis of Alzheimer’s disease. Brain regions studied were as follows: Cb, cerebellum; Hc, Hippocampus; A10 and A22, the A10 (frontal) and A22 (temporal) regions of the cerebral cortex. B, levels of DSCR1 (Adapt78) mRNA in brains of young (5 months old) and old (24 months old) F344 × BN-F1 male rats (32). C, levels of DSCR1 (Adapt78) mRNA in brain autopsy samples from eight deceased Alzheimer’s patients and eight individuals with no signs of Alzheimer’s disease. On the x axis A10 and A22 are cerebral cortex areas, Hc is the hippocampus, and Cb is the cerebellum. The average level of DSCR1 (Adapt78) mRNA production in samples from Alzheimer’s disease patients (C) is statistically significantly higher than from non-Alzheimer’s disease patients, in a ratio of 1.4:0.7 (p < 0.02). The average level of DSCR1 (Adapt78) mRNA from samples with neurofibrillary tangles is significantly higher than from samples with no tangles (D), in a ratio of 1.4:0.5 (p < 0.004). p values for all figures were tested using planned t tests (two populations) at a significance level of 0.05.
hippocampus of brains from Alzheimer’s patients was approximately double that seen in the corresponding areas of normal brains, whereas DSCR1 (Adapt78) expression in the cerebellum was similar (Fig. 2C).

Samples from patient number 9 who had no Alzheimer’s disease diagnosis (Table 1) did display DSCR1 (Adapt78) expression levels similar to the Alzheimer’s disease-affected brains. However, Bielschowsky and thioflavin-S stained sections of this particular brain revealed extensive neurofibrillary tangles that are generally associated with Alzheimer’s disease. Since neurofibrillary tangles are generally considered to be an early event in Alzheimer’s disease, it is possible that this patient had an early stage of the disease. Alternatively, high levels of DSCR1 (Adapt78) expression might be associated with neurofibrillary tangles themselves, as shown by further analysis based on the presence or absence of tangles (irrespective of Alzheimer’s diagnosis status) in Fig. 2D. DSCR1 (Adapt78) levels in the hippocampus and cerebral cortex from brains with extensive neurofibrillary tangles were three times higher than in the same regions of brains without tangles (Fig. 2D), whereas DSCR1 (Adapt78) expression in the cerebellum was similar (Fig. 2C).

DSCR1 (Adapt78) Expression in Down Syndrome—We were able to perform limited studies of post-mortem brain tissues from Down syndrome patients. Analysis of the samples from four adult patients revealed up to 2-fold higher expression of DSCR1 (Adapt78) in the A10 and A22 areas of the cerebral cortex from Down syndrome patients and up to 3-fold higher expression in the hippocampus (data not shown). These results confirm the preliminary findings of Fuentes et al. (23) that DSCR1 (Adapt78) is overexpressed in one sample of Down syndrome fetal brain as compared with the control sample.

Amyloid β-peptides Aβ42 and Aβ40 Stimulate DSCR1 (Adapt78) Expression, but Thyroid Hormone Has No Effect—Since β-Amyloid deposition is thought to play an important role in Alzheimer’s disease, we tested whether Aβ peptides could modulate DSCR1 (Adapt78) expression in the human glioblastoma/astrocytoma U-373 MG cell line. Aggregated amyloid β1–42 peptide was added to cell media at final concentrations of 0.38 μM (1.7 μg/ml), 7.6 μM (34.0 μg/ml), or 0.0 μM concentrations of Aβ1–42 peptide (a major component of human amyloid plaques) for 24 h prior to isolation of total RNA. The Aβ1–42 peptide was obtained from U. S. Peptide, Inc., dissolved in PBS, and allowed to aggregate by 37 °C incubation overnight.

We next asked whether the induction of DSCR1(Adapt78) mRNA by Aβ1–42 in a U-373 cell line. Cells were cultured at 37 °C in Eagle’s minimal essential medium supplemented with 10% fetal bovine serum and 1.0 mM sodium pyruvate, under a 5% CO2 atmosphere. They were exposed to 0.38 μM (1.7 μg/ml), 7.6 μM (34.0 μg/ml), or 0.0 μM concentrations of Aβ1–42 peptide and Aβ1–40 peptide on DSCR1 (Adapt78) expression in the human neuroblastoma cell line SK-N-MC. As in U-373 MG cells (Fig. 4), the amyloid peptides could modulate DSCR1 (Adapt78) expression by Aβ1–42 peptide and Aβ1–40 peptide in a dose-dependent manner. The Aβ1–42 peptide stimulated DSCR1 (Adapt78) mRNA expression in control cells treated with the vehicle (PBS) alone. We also analyzed the effect of Aβ1–42 peptide and Aβ1–40 peptide on DSCR1 (Adapt78) expression in SK-N-MC cells (confirmatory data not shown).

We next asked whether the induction of DSCR1(Adapt78) mRNA by Aβ1–42 in a U-373 cell line. Cells were cultured at 37 °C in Eagle’s minimal essential medium supplemented with 10% fetal bovine serum and 1.0 mM sodium pyruvate, under a 5% CO2 atmosphere. They were exposed to 0.38 μM (1.7 μg/ml), 7.6 μM (34.0 μg/ml), or 0.0 μM concentrations of Aβ1–42 peptide and Aβ1–40 peptide on DSCR1 (Adapt78) expression in SK-N-MC cells (confirmatory data not shown). DSCR1 (Adapt78) was
also not induced by incubating cells with 5 mM sodium azide as described by Hansen et al. (36); however, it was induced following exposure to 2 μg/ml sodium arsenite as described by Hei et al. (37) (data not shown). It has been reported that sodium azide causes cell death by necrosis (38), while arsenite (39) and β-amyloid (40) cause cell death by apoptosis. Our preliminary results so far may thus suggest that DSCR1 (Adapt78) might be selectively activated by toxic insults that cause cell apoptosis via calcium fluxes.

The DSCR1 (Adapt78) homologue ZAKI-4 gene was discovered as a thyroid hormone-responsive gene (41). We, therefore, also analyzed whether thyroid hormone can induce DSCR1 (Adapt78) in U-373 MG cells. The cells were grown as described in the legend to Fig. 4 and exposed to physiological levels of T3. A section of rat cerebellum was hybridized with an antisense probe to DSCR1 (Adapt78) exons 1, 5, 6, and 7. B, a section of rat cerebellum was hybridized with a sense probe to DSCR1 (Adapt78) exons 1, 5, 6, and 7. In both panels neuronal layers are marked by arrows. Magnification is ×40.

FIG. 5. Expression of DSCR1 (Adapt78) mRNA in rat brain. A, a section of rat cerebellum was hybridized with an antisense probe to DSCR1 (Adapt78) exons 1, 5, 6, and 7. B, a section of rat cerebellum was hybridized with a sense probe to DSCR1 (Adapt78) exons 1, 5, 6, and 7. In both panels neuronal layers are marked by arrows. Magnification is ×40.

DSCR1 (Adapt78) Overexpression in Alzheimer’s Disease

In the present study we report that DSCR1 (Adapt78) is highly expressed in different areas of the adult human brain. This is in agreement with previous findings that DSCR1 is expressed in adult human brain (21). It is interesting that its homologues DSCR1I1 (41) and DSCR1I2 (42) are also expressed in adult human brain. Our new detailed studies demonstrate that DSCR1 (Adapt78) is particularly expressed in those brain areas that are most affected by Alzheimer’s disease: the cerebral cortex and the hippocampus. Moreover, we analyzed DSCR1 (Adapt78) expression at the cellular level and demonstrated that it is predominantly expressed in neurons. Since DSCR1 (Adapt78) function has been associated with calcineurin inhibition, it is also interesting that the highest level of calcineurin expression in mammalian tissues is found in the brain (43–45). We now report that the level of DSCR1 (Adapt78) mRNA in those human brain areas affected by Alzheimer’s disease is approximately double that found in age-matched samples from individuals with no Alzheimer’s diagnosis (post-mortem analysis).

Interestingly, DSCR1 (Adapt78) overexpression was even more strongly associated with one of the key features of Alzheimer’s disease: development of neurofibrillary tangles in the hippocampus and frontotemporal cerebellar cortex. Recent studies indicate that abnormal phosphorylation of the tau protein, which is a major component of the tangles, can be one of the key pathways in Alzheimer’s disease development (8). Previously it was shown that decreased calcineurin phosphatase activity allows accumulation of hyperphosphorylated tau protein and cytoskeletal changes in the hippocampus that are similar to those observed during Alzheimer’s disease development (28). DSCR1 has been shown to bind to calcineurin and reduce its activity (24, 25), and it is possible that one of the mechanisms by which DSCR1 (Adapt78) can regulate tau phosphorylation involves binding to the catalytic subunit of calcineurin. Since diminished calcineurin activity has already been reported to occur in Alzheimer’s disease (31), our proposed mechanism of DSCR1 (Adapt78) regulation of tau phosphorylation may well be of pathological significance in vivo. Decreased calcineurin activity also causes complete abolishment of synaptic depletiation, indicating that calcineurin might affect learning and memory processes (30). It is possible that increased DSCR1 (Adapt78) levels in brain areas affected by Alzheimer’s disease may increase translation of the DSCR1 (Adapt78) protein, inhibit calcineurin activity, cause accumulation of hyperphosphorylated tau protein and cytoskeletal changes, produce neurofibrillary tangles, and promote the development of Alzheimer’s disease.

DSCR1 (Adapt78) is predominantly expressed in neurons,
and there are indications that the degeneration of neurons in Alzheimer's disease occurs by apoptosis (28, 29). Recently it has also been shown that calcineurin can promote apoptosis of neuronal cells (26) and therefore DSCR1 (Adapt78) might also be involved in this process by regulation of calcineurin. We can now show that toxic concentrations of Aβ1–42 stimulate DSCR1 (Adapt78) expression in an in vitro model. Since transient overexpression of DSCR1 (Adapt78) can "rescue" cells from stress-induced apoptosis (17–19) and from calcineurin-induced apoptosis (26), this is probably a protective mechanism in the short term. Thus DSCR1 (Adapt78) may be connected with the two most prominent phenomena in Alzheimer's disease: development of amyloid plaques and neurofibrillary tangles.

We must now reconcile two "apparently" contradictory DSCR1 (Adapt78) findings: protection against stress-induced apoptosis (17–19) and association with diseases such as Down syndrome (Ref. 24 and this study) and Alzheimer's disease (this study). At this early stage it seems possible that transient overexpression of DSCR1 (Adapt78) is normally used as a protection against shock or stress. In contrast, uncontrolled chronic overexpression of DSCR1 (Adapt78) may actually predispose cells to Alzheimer's disease. Since Down syndrome involves chronic overexpression of DSCR1 (Adapt78), it would be expected to cause symptoms quite early in life. It is also possible that repeated environmental stresses may trigger chronic overexpression of DSCR1 (Adapt78) and result in increased susceptibility to Alzheimer's disease. Finally, we can also hypothesize that DSCR1 (Adapt78) overexpression may be an attempt to cope with exposure to amyloid protein in the early stages of Alzheimer's disease. Although such chronic overexpression of DSCR1 (Adapt78) may protect against amyloid-induced apoptosis, it could eventually lead (via tau hyperphosphorylation) to the formation of neurofibrillary tangles and Alzheimer's symptoms.

Here we demonstrate that the level of DSCR1 (Adapt78) mRNA is not significantly changed during aging of either human or rat brains. There was no association between the age or sex of adult patients and the level of DSCR1 (Adapt78) expression in various areas of human brains. Similarly, we also did not find any significant changes in DSCR1 (Adapt78) levels in cerebral cortex or cerebellum of older (24 months old) versus young (5 months old) rats. These data also indicate that age-matching (although used in this work) was not a critical parameter in our Alzheimer's disease study. Higher expression of this gene has been reported in the cortex of neonatal rats compared with adult rats (20). The level of expression was high in the cortex of 2- and 7-day-old rats and then decreased after day 16, suggesting an important role of DSCR1 (Adapt78) in neuronal growth and development. On the other hand we observed that DSCR1 (Adapt78) overexpression in the adult brain is associated with neuronal degeneration. Taken together these findings indicate that DSCR1 (Adapt78) might be important in growth and development of young neurons, whereas deregulation of DSCR1 (Adapt78) might promote Alzheimer's disease-like degeneration in older neurons.

Binding and regulation of calcineurin might not be the only function or major function of DSCR1 (Adapt78). It was recently demonstrated that a stretch of about 80 amino acids near the N terminus of DSCR1 (Adapt78) protein family members shows similarity with an RNA recognition motif that is found on many RNA-binding proteins and in a few single-stranded DNA-binding proteins (42). The possibility that DSCR1 (Adapt78) may function as an RNA- or DNA-binding protein has not yet been properly tested.
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