Neuroscience 159 (2009) 638–646

ASYMMETRIC EXPRESSION PATTERNS OF BRAIN TRANSTHYRETIN IN NORMAL MICE AND A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

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Abstract—Brain asymmetry is linked with several neurological diseases, and transthyretin (TTR) is a protein sequestering β-amyloid (Aβ) and helping to prevent the Alzheimer's disease (AD). We show, by real time reverse transcription–polymerase chain reaction (RT-PCR), *in situ* hybridization and Western blotting, that TTR exhibits a pattern of adult male-specific, leftward distribution in the mouse brain. This asymmetry appeared to be mainly due to the asymmetric distribution of the choroid plexus cells in the ventricles. Unlike the normal mice, however, the hemispheric levels of TTR transcripts of 2- and 6-month-old Tg2576 mice, a transgenic AD mouse model overexpressing Aβ, were symmetric in both sexes. Furthermore, at the age of 10 months when the pathological AD-like features had developed, the level of TTR transcripts in the left hemisphere of the male Tg2576 became significantly lower than the right one. This lowering of TTR transcript is accompanied with a higher Aβ level in the left hemisphere of the 10-month Tg2576 males. Finally, for both genders, the TTR transcript levels in the two hemispheres of aged Tg2576 mice were lower than either the adult Tg2576 or the aged nontransgenic controls. Based on the above, we suggest scenarios to correlate the changes in the levels and hemispheric patterns of TTR expression to the pathogenesis of AD.

Key words: Alzheimer's disease, asymmetry, gene expression, mouse brain, transthyretin.

Transthyretin, or TTR, is the most abundant protein expressed in the choroids plexus of the brain (Sousa et al., 2007) in mammals, birds, and reptiles (Schreiber, 2002). This evolutionary conservation suggests important role(s) played by TTR in the mammalian brain. TTR is one of the principal carriers in the plasma for the thyroid hormones (Palha et al., 1994), which are essential for the development and function of the mammalian brain (Schreiber, 2002). TTR expressed in the choroid plexus may carry thyroid hormone T_4 into the CNS through the blood–brain barrier (BBB) (Chanoine et al., 1992). Importantly, TTR protein has been shown to bind and sequester Aβ to prevent its aggregation (Schwarzman et al., 1994; Link, 1995) thus playing a neuroprotective role in AD (Stein et al., 2004; Buxbaum et al., 2008). On the other hand, TTR gene expression could be induced in response to overproduction of Aβ (Stein and Johnson, 2002). This seems to be in contrast to the observations that the concentration of TTR is lower in most, if not all, AD patients than in age-matched controls (Serot et al., 1997; Chodobski and Szmydynger-Chodobska, 2001).

Accumulating evidence has indicated that human brain is indeed asymmetrical in anatomical, functional, as well as neurochemical aspects (Galaburda et al., 1978; Geroldi et al., 2000b, Hugdahl, 2000, Toga and Thompson, 2003). Differences between the left and right hemispheres have been implicated in many aspects of the brain function and dysfunction, including the developmental disorders of schizophrenia, epilepsy, dyslexia and AD (Barta et al., 1997; Geroldi et al., 2000a). It has also been hypothesized that failure to develop proper asymmetry is an important factor of the pathology underlying some forms of neurological diseases (Geschwind and Miller, 2001; Toga and Thompson, 2003). In particular in patients with AD, for example, loss of asymmetry in the hippocampus has been observed (Geroldi et al., 2000b). In addition, the atrophy of the right parahippocampal gyrus is deteriorated in AD patients (Pantel et al., 2003). In those AD patients with delusions, a reversed pattern of temporal and frontal horn asymmetries were found (Geroldi et al., 2000a).

The presence of brain asymmetries in mammals other than human (Tabibnia et al., 1999; Sun and Walsh, 2006) opens the possibility for molecular and cellular research on the structure-function of brain asymmetry by using animals such as mouse as the model system (Kawakami et al., 2003; Sun et al., 2005). As shown below, we have identified TTR as a gene exhibiting an asymmetric expression pattern of its transcripts in the male mouse brains, but not in the females. Interestingly, this pattern of asymmetric TTR expression was altered in aged mice as well as in a transgenic mouse model of AD.

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Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, β-amyloid; BBB, blood–brain barrier; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HE, hematoxylin and eosin; IMB, Institute of Molecular Biology; RT-PCR, reverse transcription–polymerase chain reaction; TTR, transthyretin.

0306-4522/09
doi:10.1016/j.neuroscience.2008.12.045

EXPERIMENTAL PROCEDURES

Animals

Eight-week-old C57BL/6JNarl mice were purchased from the National Laboratorial Animal Center (Taipei, Taiwan), while transgenic Tg2576 mice and non-transgenic littermates were purchased from Taconic (Germantown, NY, USA). The mice were bred at the Animal Facility of the Institute of Molecular Biology (IMB), Academia Sinica, Taiwan. They were housed in a room maintained on a 12-h light/dark cycle (light on at 7.00 AM) with continuous supply of food and water. The use of animals in this study was approved by the Institutional Animal Care and Use Committee in IMB. Experimental procedures for handling the mice also followed the guidelines of IMB. The authors attest that all efforts were made to minimize the number of animals used and their suffering.

Tissue preparation

Each mouse was perfused with 20 ml 0.1 M PBS and 20 ml 4% paraformaldehyde/0.1 M PBS. The brain was taken out immediately and incubated in 4% paraformaldehyde/0.1 M PBS for an hour, and then soaked in 20% sucrose/4% paraformaldehyde for at least 1 day.

RNA preparation

The left and right cerebral hemispheres were dissected from normal C57BL/6JNarl mice or the Tg2576 AD mice at different ages. They were lysed in TRIzol REAGENT (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. After homogenization, the homogenates were extracted with chloroform and isopropanol, and precipitated in ethanol. The final RNA solutions were further cleaned up using the RNeasy kit (QIAGEN, Valencia, CA, USA) as well as with a DNase treatment step to remove the contaminated DNA. The concentrations of the RNAs were determined by Ultraspec 3000 spectrophotometer (Amersham Pharmacia Biotech, Piscataway, NJ, USA), and their qualities were validated by measuring the ratios of the 28 S rRNA/18 S rRNA in an Agilent (2100) BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA).

DNA microarray hybridization

The RNAs of the left and right hemispheres, respectively, of two adult male mice were isolated. Microarray hybridization experiments were then carried out, each using 20 μg of the RNAs from the left and right hemispheres, respectively. The probe labeling and hybridization were carried out at the DNA MicroArray Facility of the University of California, Irvine. In brief, the RNA samples were reverse transcribed into double-stranded cDNA using the Superscript Choice kit (Invitrogen, Carlsbad, CA, USA) containing a T7-DT_{24} primer that would incorporate a T7 RNA polymerase promoter during reverse transcription. The CRNAs were biotin-labeled during *in vitro* transcription (Enzo Biochem, New York, NY, USA), and used as the probes for hybridization with the MG-U74Av2 arrays containing 12,473 oligo probe sets (Affymetrix, Santa Clara, CA, USA). After hybridization, the gene chips were washed and stained with streptavidin–phycoerythrin on a fluidics station. Finally, the probed arrays were scanned at 3 μm resolutions in the Genechip System Confocal Scanner (Affymetrix, Santa Clara, CA, USA). Affymetrix Microarray Suite 4.1 was used to scan and analyze the relative abundance of each gene based on the average differences of the intensities (Lipshutz et al., 1999). The candidate genes were selected based on two criteria: First, the signals must be called as "present" in as least one set of the samples; second, the change of the expression level must be determined as "increase" with a signal log ratio ($\log_2$L/R) above 0.1, or as "decrease" with a signal log ratio below −0.1. Finally, the definitions of "increase," "decrease," or "no change of expression" for the individual genes were based on the three intergroup comparisons between the duplicate hybridization experiments.

RT-PCR

Both real time RT-PCR and semi-quantitative RT-PCR were used to compare the mRNA levels. For each reaction of first-strand cDNA synthesis, 0.5 μg of the total RNA were mixed with 500 ng of oligo $(dT)_{18}$ and 0.5 mM dNTP mixture. The mixture was heated to 65 °C for 5 min and quickly chilled on ice before addition of the 5× first-strand buffer, 10 mM DTT, and 40 U of ribonuclease inhibitor (Promega, Madison, WI, USA) in a total volume of 19 μl. The mixture was incubated at 42 °C for 2 min, added with 1 μl of SuperScript II (Invitrogen), and then incubated for another 70 min at 42 °C. Ten-fold diluted cDNAs were used as the templates for PCR.

The real time PCR was then performed with 25 ng of cDNA in an Applied Biosystems (AB, Foster City, CA, USA) 7500 Sequence Detection System using the AB Taqman Gene Expression Assays for TTR (Mm00443267-m1, the amplicon length of which was 62 bp and its boundaries were from exon 3 to exon 4) and primers-limited glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Mm99999915-g1), respectively. No significant differences in GAPDH expression were detected among the different groups of samples. The threshold amplification cycle number (ct) data from multiple plates were combined using AB Relative Quantitation software (SDS1.4) and the ΔΔCt method with GAPDH as the endogenous control. All data were expressed as the mean fold-of-change ±SE.

For semi-quantitative PCR, the forward and reverse primers used for TTR were 5′-CCT GCT CAG CCC ATA CTC CTA-3′ and 5′-AAC GGT TGG TCC ACT CTG CT-3′, respectively, both of which were derived from exon 4. The primers for GAPDH were 5′-GAC CCC TTC ATT GAC CTC AAC-3′ and 5′-TCT TAC TCC TTG GAG GCC ATG-3′. The PCR reactions were initiated by denaturation at 94 °C for 3 min, followed by 32 cycles at 94 °C for 30 s, 60 °C for 30 s, and finally at 72 °C for 30 s. The PCR products were allowed to be extended at 72 °C for 10 min, and the products were resolved on 1.5% agarose gels. The images were captured and densitometry was performed by using the Alpha Imager 2200 (Alpha Innotech Corporation, San Leandro, CA, USA). The TTR levels were then compared after calibration against the corresponding GAPDH levels.

In situ hybridization

In situ hybridization was performed according to the procedures described previously (Tsai et al., 2002) with slight modifications. Brain coronal sections with the width of 20 μm were taken from the forebrain to the hind-brain serially, covering the hippocampus and the ventricles (central and lateral) of the cerebrum. The antisense probe complementary to nucleotide 307–351 of the TTR cDNA (5′-CAT GGA ACG GGG AAA TGC CAA GTG TCT TCC AGT ACG ATT TGG TGT-3′) and the sense probe were 3′ end-labeled with α[^{35}S]dATP and hybridized, respectively, at 42 °C for 24 h with the silane-coating slides (Muto Pure Chemicals Co., New York, NY, USA) containing at least 50 brain sections. After extensive washing, the slides were dehydrated with ethanol and exposed to Hyperfilm MP (Amersham Pharmacia Biotech, Piscataway, NJ, USA) for 10 days. The signals from the *in situ* hybridization were quantified by measuring the optic densities of the relevant fields with the use of the National Institutes of Health Image program.

Western blotting

The left and right hemispheres of the male and female mice at the age of 2 months were homogenized in RIPA lysis buffer [Tris–HCl 50 mM, NaCl 150 mM, Igepal CA-630 1%, EDTA (pH 8.0) 2 mM,