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Human apoB overexpression and a high-cholesterol diet differently modify the brain APP metabolism in the transgenic mouse model of atherosclerosis

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Abstract

Epidemiological and biochemical data suggest a link between the cholesterol metabolism, the amyloid precursor protein (APP) processing and the increased cerebral β-amyloid (Aβ) deposition in Alzheimer's disease (AD). The individual and combined effects of a high-cholesterol (HC) diet and the overexpression of the human *apoB-100* gene were therefore examined on the cerebral expression and processing of APP in homozygous apoB-100 transgenic mice [Tg (apoB$^{+/+}$)], a validated model of atherosclerosis. When fed with 2% cholesterol for 17 weeks, only the wild-type mice exhibited significantly increased APP695 (123%) and APP770 (138%) mRNA levels in the cortex. The HC diet-induced hypercholesterolemia significantly increased the APP isoform levels in the membrane-bound fraction, not only in the wild-type animals (114%), but also in the Tg apoB$^{+/+}$ group (171%). The overexpression of human *apoB-100* gene by the liver alone reduced the brain APP isoform levels in the membrane-bound fraction (78%), whereas the levels were increased by the combined effect of HC and the overexpression of the human *apoB-100* gene (134%). The protein kinase C and β-secretase protein levels were not altered by the individual or combined effects of these two factors. Our data indicate that the two atherogenic factors, the HC diet and the overexpression of the human *apoB-100* gene by the liver, could exert different effects on the processing and expression of APP in the mice brain.

Keywords: Alzheimer's disease; Cholesterol; ApoB-100; Transgenic mice; Amyloid precursor protein; mRNA; Brain

1. Introduction

Hypercholesterolemia, a high serum low-density lipoprotein (LDL) cholesterol level and inheritance of the ε4 allele of the *apolipoprotein E* (*apoE*) gene are all associated with atherosclerosis and both Alzheimer's disease (AD) and vascular dementias. Animals fed with a high-cholesterol (HC) diet display a tendency to accumulate β-amyloid (Aβ) in the brain (Sparks, 1996; Sparks et al., 2002). Furthermore, diet-induced hypercholesterolemia and atherosclerosis are both associated with β-amyloidosis in the transgenic (Tg) mouse model of AD (Li et al., 2003). The elevated cholesterol content of the cell membrane facilitates the β- and γ-cleavage of amyloid precursor protein (APP) in cultures of rat hippocampal neurons (Simons et al., 1998). Additionally, there is evidence that cholesterol-lowering drugs can reduce the prevalence of human AD (Wolozin et al., 2000; Zandi et al., 2005) and in Tg animal models of this type of dementia (Kirsch et al., 2003). Besides apoE, apolipoprotein B-100 (apoB-100) has also been shown to modulate cerebral Aβ deposition in vivo (Sabbagh et al., 2004).

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ApoB-100, the major vehicle for cholesterol transport in the periphery, is synthesized in the liver and small intestine, but not in the brain (Pitas et al., 1987). ApoB-100 is the key component in all classes of lipoproteins, including LDL, intermediate-density lipoprotein and very low-density lipoprotein remnants considered to be atherogenic (Kim and Young, 1998). The concentration of apoB-100 is elevated in the serum of AD patients (Caramelli et al., 1999) and correlates with the amount of cerebral Aβ deposition (Sabbagh et al., 2004). Further, abnormal levels of apoB-100 and cholesterol accumulate in the brain of AD patients and have been found as core components of the mature plaques (Namba et al., 1992; Houlden et al., 1995; Mori et al., 2001; Puglielli et al., 2003).

The serum lipid profile in mice is very different from that in humans. These rodents carry most of the cholesterol on high-density lipoprotein (HDL) and, since they have low LDL cholesterol levels, they are protected against hypercholesterolemia and do not develop atherosclerosis without dietary or genetic manipulations (Breslow, 1996). Among the inbred mouse strains, the strain C57B6 has been found to be the most susceptible to the development of atherosclerotic lesions upon administration of a HC diet (Jawien et al., 2004). We report here that human apoB overexpression and a high-cholesterol diet, either individually or in combination, exert different effects on the brain APP metabolism in this well-characterized animal model of atherosclerosis.

2. Experimental procedures

2.1. Materials

The substances used in this study were: Standard Lipid Controls from Sentinel (Milano, Italy), cholesterol colorimetric assays from Diagnosticum Ltd. (Budapest, Hungary), *Taq* DNA polymerase and the RevertAid™ First Strand cDNS Synthesis Kit from Fermentas (St. Leon-Rot, Germany), Trizol reagent, BioMax light autoradiographic film, ethylenediaminetetraacetic acid (EDTA), leupeptine, pepstatine A, phenylmethanesulfonyl fluoride (PMSF) from Sigma–Aldrich, (Sigma, St. Louis, MO, USA), sodium dodecylsulfate (SDS) from Merck (Darmstadt, Germany), anti-Alzheimer precursor protein A4 (Mab 22C11), anti-protein kinase C (anti-PKC), β-secretase (anti-BACE) polyclonal antibody from Chemicon International Inc. (Temecula, CA, USA), HMW-SDS marker kit and Hybond ECL nitrocellulose membrane from Amersham Pharmacia Biotech (Uppsala, Sweden), HRP-conjugated anti-mouse IgG and HRP-conjugated anti-rabbit IgG from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA, USA); Tween 20 from ICN Biomedicals (Eschwege, Germany); and Supersignal® West Pico Chemiluminescent Substrate from Pierce (Rockford, IL, USA). All other reagents were commercial products of analytical grade with the highest purity available.

2.2. Tg animals and diet

Fertilized oocytes from wild-type female mice (C57/B6 × CBA F1) were collected and injected with purified P1-phagemid DNA containing the entire 43 kb human *apoB-100* gene, the 19 kb of the 5′ and the 14 kb of the 3′ flanking genomic sequences (kind gifts of Prof. Rubin, Berkeley Laboratory, University of California, Berkeley, CA, USA) (Callow et al., 1994) in a concentration of 1 ng/ml, according to a standard technique (Hogan et al., 1996). The apoB transgenic mice [Tg ($apoB^{+/+}$)] were homozygous for the human apoB transgene. Both the wild-type F1 and the Tg F1 generation mice lines were backcrossed twice with the C57B6 strain in order to approach closer to the susceptible genetic background. The wild-type animals were not littermates of the Tg ($apoB^{+/+}$) mice.

The tail DNA of 10-day-old pups was purified (Sambrook et al., 1989), and integrated transgenes were detected by PCR, using primers from the 5′ promoter region of the human *apoB-100* gene (Callow et al., 1994). The best-expressing line (No. 485) was selected for further studies. Only female animals were used because this gender is considered to be a better model of atherosclerosis than males since they furnish more and larger atherosclerotic lesions (Kim and Young, 1998). The 6-week-old wild-type and Tg ($apoB^{+/+}$) mice were fed with a standard laboratory diet supplemented or not with 2% cholesterol (five mice/group) for 17 weeks under standard laboratory conditions (Csont et al., 2002). The animals were anesthetized after the treatment period and blood samples were obtained intracardially. Brains were removed without perfusion and the whole cerebral cortex was isolated and stored at −70 °C until used. The experimental protocol was approved by the local animal care ethical committee.

2.3. Determination of serum lipid and cholesterol levels

Serum lipids, total cholesterol and LDL and HDL cholesterol levels were measured in triplicate, using commercially available colorimetric assays adapted to a 96-well plate kit. HDL cholesterol was measured following the direct immunological inhibition of β-lipoproteins. LDL cholesterol was also determined following the selective protection of LDL. The accuracy of the assays was monitored by using Standard Lipid Controls. Results are expressed as mM/l of serum.

2.4. Reverse transcriptase polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted from the brain cortex with the guanidium thiocyanate phenol/chloroform method (Chomczynski and Sacchi, 1987). Five micrograms of total mRNA was transcribed into cDNA with oligo $(dt)_{18}$ primers (RevertAid™ First Strand cDNS Synthesis Kit from Fermentas). The cDNA was amplified by PCR with oligonucleotides for β-actin, APP770 and APP695 (539, 401 and 242 bp of PCR products, respectively). Primer sequences are shown in Table 1. Reactions were performed with a My Cycler™ Thermal Cycler (BioRad, CA, USA). PCR was carried out in a final volume of 25 μl, containing 2.5 μl of 10× PCR buffer, 2 μl of 25 mM $MgCl_2$, 4 μl of 5 mM dNTP, 3 pmol of primers specific for β-actin and APP695, and 10 pmol of primers for APP770, 2 μl of the template cDNAs described above and 0.25 μl of *Taq* DNA polymerase (5 U/μl). Thirty cycles consisted of denaturation (30 s at 95 °C), annealing (30 s at 55 °C) and extension (1 min at 72 °C). PCR products were separated by 1.3% agarose gel electrophoresis. The ratios APP695/β-actin and APP770/β-actin mRNA were calculated.

2.5. Real-time RT-PCR (QRT-PCR)

The expression of the human *apoB-100* transgene was detected by using real-time RT-PCR. The total RNA from the liver of transgenic mice was purified with Trizol reagent and reverse transcribed (RevertAid First Strand cDNA Synthesis Kit). Primer sequences for QRT-PCR are shown in Table 1. PCR reactions were run on a Rotor-Gene 2000 (Corbett Research, Sydney, Australia), using SYBR green dye to detect double-stranded products. Curves were analyzed by Rotor-Gene software, using dynamic tube and slope correction methods, data from cycles close to the baseline being ignored. Relative expression ratios were normalized to β-actin. The analysis of the results was performed by the Pfaffl method (Pfaffl, 2001).

2.6. Sample preparation for Western blotting

For APP and PKC detection, five samples from each group were homogenized in 50 mM Tris buffer, pH 7.5, containing 0.15 M NaCl, 2 mM PMSF, 2 mM EDTA, 2 μg/ml leupeptin and 1 μg/ml pepstatin by using a glass–Teflon potter (1500 rpm, 1 min). The cortical homogenates were centrifuged at 100 000 × *g* for 30 min at 4 °C. The supernatant represented the soluble fraction, while the pellet was resuspended in the same volume of buffer containing 1% SDS and centrifuged as before. The new supernatant represented the membrane fraction.

For BACE measurement, the soluble and membrane-bound fractions were not separated. Cortices were homogenized in 50 mM Tris buffer pH 7.5