

Supplementary Data

Processing of Endogenous A β PP in Blood-Brain Barrier Endothelial Cells is Modulated by Liver-X Receptor Agonists and Altered Cellular Cholesterol Homeostasis

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ANTIBODIES

Rabbit polyclonal anti-A β PP antibody (ab) directed against the C-terminus of human A β PP was purchased from Invitrogen (Vienna, Austria; product number 51–2700) and was used for immunoblotting to detect mature and immature A β PP (m-A β PP and im-A β PP, 1 : 1500 dilution). The A11 rabbit polyclonal anti-Amyloid Oligomer ab directed against a peptide backbone that is common to amyloid oligomers was purchased from Millipore (Vienna, Austria) and was used for immunoblotting to detect intra- and extracellular A β oligomers (1 : 10,000 dilution). The BAM-10 monoclonal mouse-anti β -Amyloid ab specifically recognizes amino residues 1–12 of A β peptide, cor-

responding to aa 672–683 in human/porcine A β PP. It was purchased from Sigma Aldrich (Vienna, Austria) and was used for immunoblotting to detect A β and secreted, soluble A β PP alpha (sA β PP α , 1 : 1500 dilution). Rabbit polyclonal anti-sA β PP β ab recognizes the soluble fragment cleaved N-terminal to the beta-secretase cleavage site of A β PP, was purchased from Covance (Anopoli, Vienna, Austria) and was used for immunoblotting to detect secreted, soluble A β PP beta (sA β PP β , 1 : 200). Rabbit polyclonal anti-HMG-CoA reductase (HMGCR) ab detects an epitope within amino residues 550–650 of human HMGCR, was purchased from Abcam (Vienna, Austria) and was used for immunoblotting to detect HMGCR (1 : 1000 dilution). Rabbit polyclonal anti-sterol regulatory element binding protein 2 (SREBP-2) ab detects an epitope within amino residues 300–400 of human SREBP-2, was purchased from Abcam and was used for immunoblotting to

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detect SREBP-2 (1 : 1000 dilution). Rabbit polyclonal anti-ATP-binding cassette transporter A1 (ABCA1) ab detects an epitope within amino residues 1200–1300 of human ABCA1, was purchased from Abcam and was used for immunoblotting to detect ABCA1 (1 : 1000 dilution). Rabbit polyclonal anti- β -amyloid ab specifically recognizes amino residues 693–706 of human A β PP protein and was purchased from Sigma Aldrich. It was used for immunohistochemistry and immunofluorescence (1 : 200 dilution) to detect A β PP in cerebral vessels and brain capillary endothelial cells, respectively. Rabbit polyclonal anti-von Willebrand factor (vWF) ab detects human vWF, was purchased from DAKO (Vienna, Austria), and was used for immunohistochemistry to detect vWF

in cerebral vessels (1 : 10,000 dilution). Rabbit polyclonal anti-apoA-I was a gift from Dr. E. Malle (Institute of Molecular Biology and Biochemistry, Medical University of Graz, Austria). Rabbit polyclonal anti- β -actin ab detects the C-terminal residues Ser-Gly-Pro-Ser-Ile-Val-His-Arg-Lys-Cys-Phe of the β -actin protein, was purchased from Sigma Aldrich and was used for immunoblotting to detect β -actin (1 : 5000 dilution). Secondary antibodies conjugated to horseradish peroxidase used for immunoblotting were either goat polyclonal to rabbit IgG purchased from Abcam (1 : 10,000 dilution) or goat polyclonal to mouse IgG purchased from Sigma Aldrich (1 : 6000 dilution).

Supplementary Table 1
Primer sequences for quantitative real-time PCR

HPRT-1	AGGACCTCTCGAAGTGTGG CAGATGGCCACAGGACTAGA	247
ACAT-1	GCCACTAAGCTTGGTTCCAT GCTTGTCCCTTCACCTCCTTG	118
ACAT-2	CATAGAAGCCATGTCCAAGC ACATCTCCAGTGACCAACC	264
SREBP-2	GCTTCTCCCCCTACTCCATC GAGAGGCACAGGAAGGTGAG	151
HMGR	CTTGTTACGCGCACAGTCG GACAGCCAGAAGGAGAGCCA	207
ABCA1	GCCATTCTCCGGGCAAC GGCTTCACGCCGCTGAT	252
PSEN1	CATGACTATTCTCCTGGTGG GCAATCATTCTACCACACC	201
PSEN2	CCTCCTCAACTCCGTGCTCA GGTAGTCCATGGCCACGTTG	203
NCSTN	GGTCTCCTTCGCCTTCTGTC GCTCTCCAGCAGAACCATGT	255
PSENE1	ATGAACCTGGAGCGAGTGTC CTCTGTCTGTGTAGGCTGG	152
APH-1a	GCCTCTGTGGTCTGGTTCAT ATCTTCGGTCCCTCACTCAGC	184
ADAM10	AGCAACATCTGGGGACAAAC CTTCCCTCTGGTTGATTGC	219
BACE1	ACAGTGGCACCACCAACCTT GCCAGAAACCATCAGGGAAC	105
COX2	CCCTTCCAACTAGGCTTCC CGTAATGATGGAAGGGCAAT	237
TNF α	CCACCAACGTTTTCCTCACT CCAAAATAGACCTGCCCAGA	247
APP	GTGAAGATGGATGCGGAGTT GTGATGACAATCACGGTTGC	152