Journal of Alzheimer's Disease 20 (2010) 1–2
IOS Press

Supplementary Material

Peroxisome Proliferator-Activated Receptor Gamma Enhances the Activity of an Insulin Degrading Enzyme-Like Metalloprotease for Amyloid-β Clearance

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EXPERIMENTAL PROCEDURES

The primer sequences used for qPCR experiments are detailed in the table below:

Gene	Primer forward	Primer reverse
IDE	5'CTCGGAACCTTGCTTCAACAC3'	5'GGCCCGCTGAAGACGATA3'
NEP	5'CCCGTTACGGCAACTTTGAC3'	5'TTTTGGGTTCTTGAAGGACATCTT3'
ECE1	5'CGTTGGGCCCCATGTTT3'	5'ATCTCGGTGGCTATGCTCTTG3'
ACE	5'GGAAGCATCACCAAGGAGAACTATA3'	5'GCAGAGGCCCTGGTACTTCA3'
MMP9	5'CCCTGGAGACCTGAGAACCA3'	5'AACCATAGCGGTACAGGTATTCCT3'
Cathepsin B	5'TCAACTATGTCAACAAACGGAATACC3'	5'CAAGTAGCTCATGTCCACGTTGTA3'
TPA	5'TGACGTGGGAGTACTGTGATGTG3'	5'AACTGAGGCTGGCTGTACTGTCT3'
UPA	5'GCGCTGACACGCTTGCT3'	5'CACCTGCCCTCCTTGGAA3'
ABCA1	5'CTCCCGGAGTTGTTGGAAAC3'	5'AAGCCTCCGAGCATCTGAGA3'

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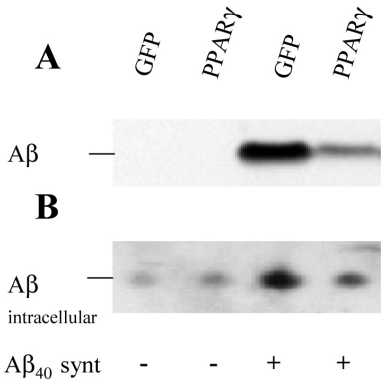
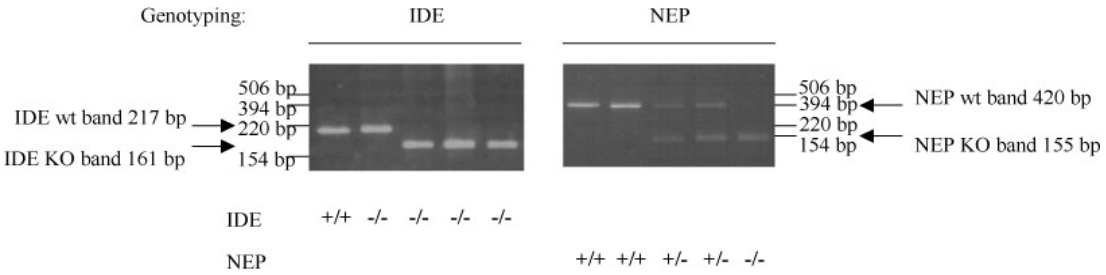
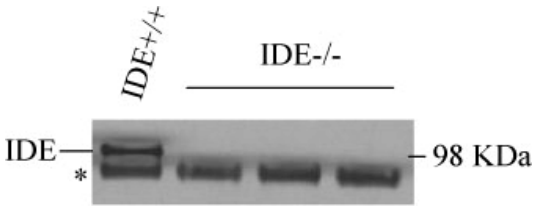
ISSN 1387-2877/10/$27.50



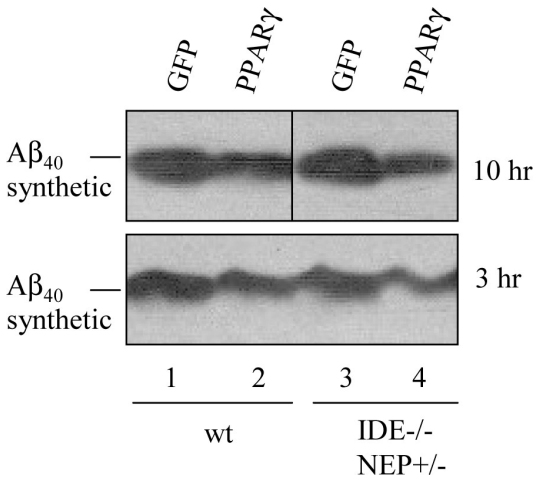
Supplementary Fig. 1. Extracellular and intracellular Aβ detection in HEK293 cells with or without synthetic Aβ incubation. A,B) Extracellular and intracellular Aβ detection from HEK293 cells expressing GFP or PPARγ incubated with or without 0.1 μM synthetic Aβ_{1-40} peptide for 3 h. Samples were analyzed by western blot for extracellular Aβ using WO2 antibody (A), and by a combination of immunoprecipitation (B7/8 antibody) and immunoblotting (WO2 antibody) from total cell extracts (RIPA buffer) for intracellular Aβ (B).



Supplementary Fig. 2. Genotyping of mouse embryos used for primary glial and hippocampal cultures. The experimental procedure and the sequence of primers used has been described before [1]. Left and right panels show the results for IDE and NEP genotyping, respectively.



Supplementary Fig. 3. IDE immunoblot detection from murine primary IDE+/+ and IDE-/- cultures. Cell extracts (prepared as described in experimental procedures) were immunoblotted using the polyclonal antibody (IDE-1) [2]. The upper band (110 Kda) corresponds to the described IDE mobility. *The lower band is unspecific [1].



Supplementary Fig. 4. Analysis of Aβ-degradation at shorter incubation times in primary hippocampal cultures derived from wild-type and IDE knock out animals. A,B) GFP- or PPARγ-transduced murine primary hippocampal cultures from wild-type (lanes 1–2) or IDE$-/-$NEP$+/-$ (lanes 3–4) were incubated with Aβ_{1-40} synthetic peptide (0.1 μM) for 10 or 3 h period. Aβ stability was analyzed by western blot using the WO2 antibody.

References

[1] Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, Eckman CB, Tanzi RE, Selkoe DJ, Guenette S (2003) Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. *Proc Natl Acad Sci U S A* **100**, 4162-4167.

[2] Vekrellis K, Ye Z, Qiu WQ, Walsh D, Hartley D, Chesneau V, Rosner MR, Selkoe DJ (2000) Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. *J Neurosci* **20**, 1657-1665.