

Supplemental data

Impact of 27-Hydroxycholesterol on Amyloid- β Peptide Production and ATP-Binding Cassette Transporter Expression in Primary Human Neurons

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REGULATION OF A β PEPTIDE GENERATION BY ABCA1 AND ABCG1 BUT NOT ABCA2

CHO-A β PP cells were transfected with human ABCA1, ABCG1 and ABCA2 cDNAs and cholesterol efflux and extracellular A β peptide levels were measured. ABCA2, which is expressed in neurons but does not respond to LXR activity or mediate cholesterol efflux, was used as a negative control in this study. The transfection efficiency of these three cDNAs was first checked by PCR and all three genes were similarly expressed (Supplemental Fig. 1A). They were then assessed for their ability to mediate cholesterol efflux as a test for their functional activity. ABCA1 and ABCG1, but not ABCA2, promoted cholesterol efflux as expected

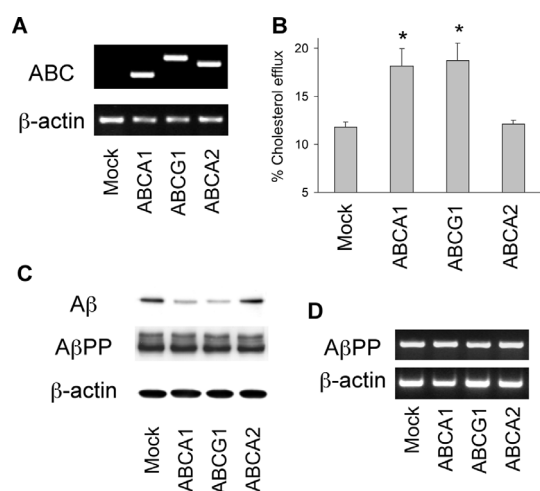
(Supplemental Fig. 1B). ABCA1 and ABCG1 (not ABCA2) reduced A β peptide levels without altering A β PP expression (both mRNA and cellular protein) indicating that ABCA1 and ABCG1 reduce extracellular A β peptide levels independently of A β PP expression (Supplemental Fig. 1C, D).

MATERIALS AND METHODS

Materials

Human ABCA1, ABCG1 and ABCA2 cDNAs were generously provided by Professor Mason Freeman (Harvard Medical School), Professor Wendy Jessup (University of New South Wales, Australia) and Professor Kenneth Tew (Medical University of South Carolina), respectively.

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Supplemental Fig. 1. Impact of ABCA1 and ABCG1 on A β peptide generation. CHO-A β PP cells were transfected with ABCA1, ABCG1, ABCA2 and empty vector (mock) cDNA and (A) the transfection efficiency was measured by PCR and agarose gel electrophoresis; (B) the ability to efflux cholesterol assayed; (C) the impact on A β peptide generation and A β PP expression analyzed by Western blotting; and the mRNA levels of A β PP measured by PCR. Data represent mean $\pm$ SE from individual experiments performed in triplicate. * $P < 0.05$.

Transfection

Transient transfection was performed using Lipofectamine 2000 and Opti-MEM I (Invitrogen) following the manufacturer's protocol. Briefly, cells were seeded at 90% confluence in 12-well plates using antibiotic-free medium. cDNA-Lipofectamine complex was added to the cells and after 24 h of incubation

samples were collected for gene expression analysis. In the case of cholesterol efflux assays the cells were cultured for up to an additional 24 h.

Cholesterol efflux assay

Cellular cholesterol efflux was measured as described previously [1]. Briefly, cells were labeled with 2 μ Ci/ml [3 H]cholesterol (Amersham Biosciences) for 24 h, rinsed with PBS, and incubated for 2 h in medium containing 0.1% (w/v) bovine serum albumin (BSA) to allow equilibration of [3 H]cholesterol in intracellular pools. The cells were rinsed once more in PBS and then incubated in serum-free medium containing apoE disc (15 μ g of protein/ml) as cholesterol acceptor. After 24 h medium samples were collected and cleared of any cellular debris by centrifugation. The cells were lysed with 0.1 M NaOH and radioactivity in the medium samples and cell lysates were measured by scintillation counting. Cholesterol effluxed to the medium was calculated as a percentage of total radioactivity in the cell lysates and medium.

SUPPLEMENTAL REFERENCE

- [1] Kim, W.S., Suryo Rahmanto, A., Kamili, A., Rye, K.A., Guillemain, G.J., Gelissen, I.C., Jessup, W., Hill, A.F. and Garner, B. (2007). Role of ABCG1 and ABCA1 in regulation of neuronal cholesterol efflux to apolipoprotein-E discs and suppression of amyloid-beta peptide generation. *J Biol Chem* **282**, 2851–2861.